NOVEL REACTIONS OF CEPH-3-EMS

Jacqueline M Torrance B.Sc. (Hons)

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requirements of the University of Abertay Dundee
for the degree of Doctor of Philosophy

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I certify that this thesis is the true and accurate version of the thesis approved by the examiners.

Signed .. [redacted] ..... Date 27-9-95

(Director of Studies)

DECLARATION

I hereby declare that the work presented in this thesis was carried out by me at University of Abertay Dundee, Dundee, except where due acknowledgement is made, and has not been submitted by me for any other degree.

Signed [redacted].....

Date .....23/6/95.....

CONTENTS

ACKNOWLEDGEMENTS

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I wish to express my sincere gratitude and appreciation to my supervisor Dr. David H Bremner for his endless encouragement, advice and motivation throughout this research. My gratitude also goes to Dr. Alan Dunn, Dr. Robert Cargill and Dr. Neil Ringan for their assistance and inspiration.

Special thanks are due to Uniroyal, Newbridge and Gavin McClement for the provision of computers to complete the typing of this thesis.

Finally, I wish to thank my family and friends for their unfailing support throughout this work.

ABSTRACT

Michael additions and reactions with haloalkanes have demonstrated the synthetic usefulness of the C-2 and C-4 anions in ceph-3-em sulphides and sulphoxides for the formation of a range of novel compounds (A-H). Other compounds prepared from similiar reactions include (I-O).

A reverse Michael addition was attempted on the C-4 mono-adducts (M) and (N) and di-adducts (L) and (O).

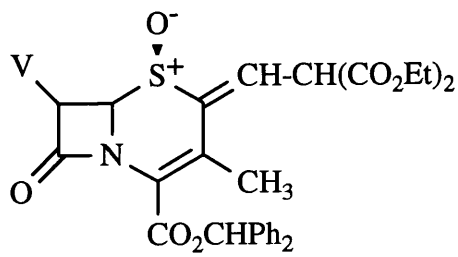
Determination of the configuration of the di-adducts (F) and (I) was accomplished by de-esterification followed by decarboxylation and resulted in the novel ceph-3-em (P).

Conversion of (J) and (K) into novel ceph-3-ems (Q) and (R) bearing C-2 exocyclic bond systems via a Pummerer rearrangement is described. Attempts at a Pummerer rearrangement of (D) and (E) failed.

Numerous attempts at a Cope rearrangement on ceph-2-em sulphoxide (G) and sulphide (H) failed.

De-esterification reactions of the new ceph-3-ems to their corresponding acids were unsuccessful.

V = $PhOCH_2CONH$



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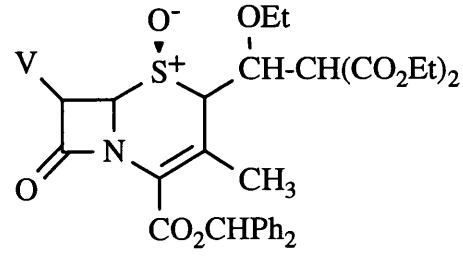

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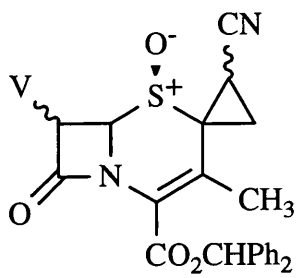

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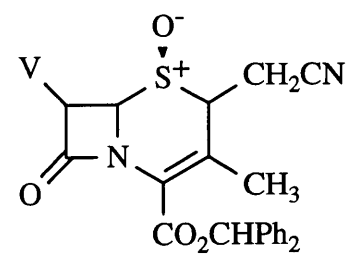

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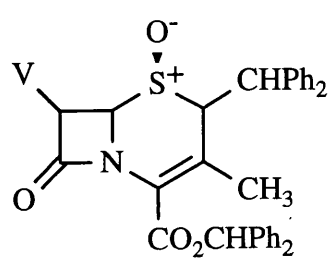

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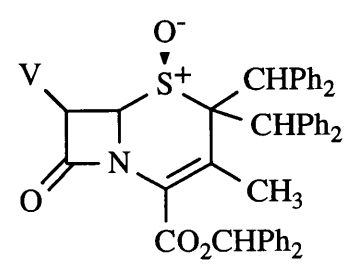

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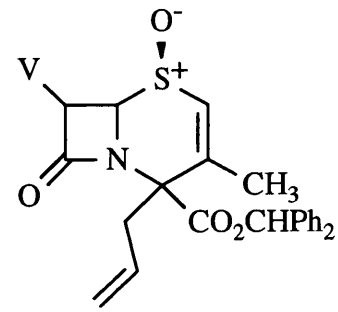

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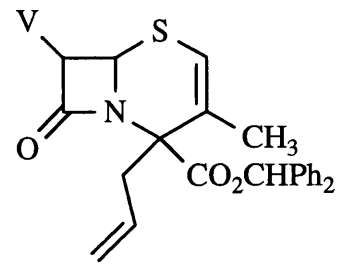

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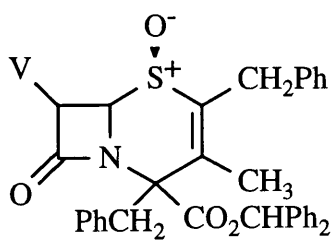

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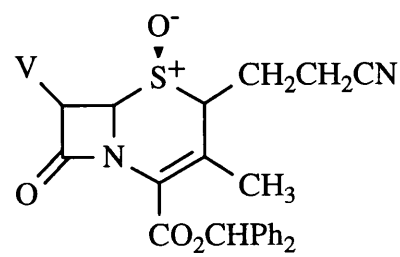

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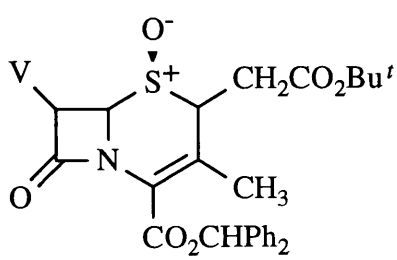

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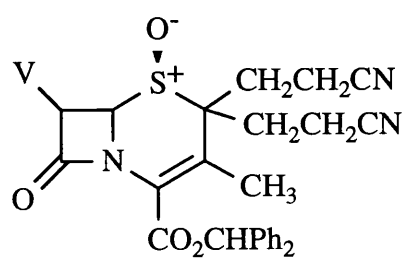

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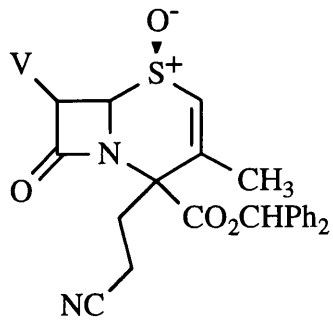

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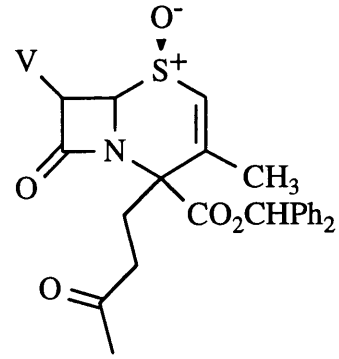

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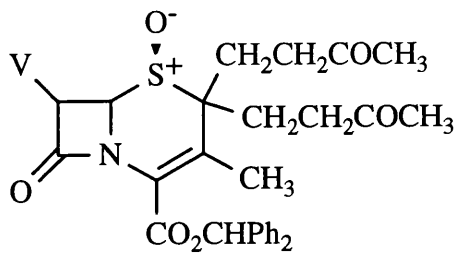

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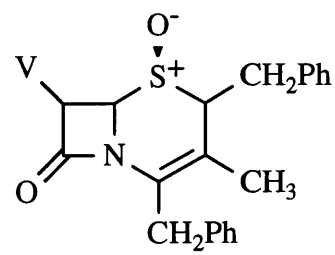

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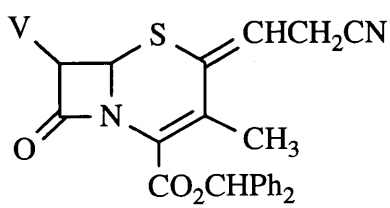

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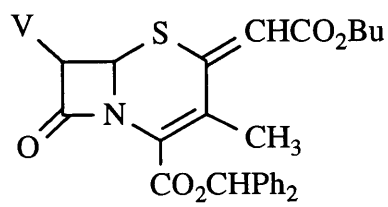

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FOREWORD

Bracketed Arabic numerals in the text refer to the diagrams of the formulae and the Arabic superscripts indicate references. The following abbreviations have been used in the text.

Ac	acetyl group (CH_3CO)
AIBN	azobisisobutyronitrile
amu	atomic mass units
Ar	aryl group
Bu^t	tertiary butyl
Bz	benzyl group
CAN	ceric ammonium nitrate
m-CPBA	*meta*-chloroperoxybenzoic acid
δ	parts per million
DBU	1,8-diazabicyclo[5.4.0.]undec-7-ene
DCC	1,3-dicyclohexylcarbodiimide
DMF	N,N-dimethylformamide
DMSO	dimethyl sulphoxide
DPM	diphenylmethyl group
DPPA	diphenylphosphoryl azide
Et	ethyl group
HLE	human leukocyte elastase
HSAB	hard soft acid base
ir	infrared
LDA	lithium diisopropylamide
Me	methyl group
mmol	millimole

mp	melting point
NBS	N-bromosuccinimide
NCS	N-chlorosuccinimide
nmr	nuclear magnetic resonance
NOE	nuclear Overhauser enhancement
Ph	phenyl group
PNB	*para*-nitrobenzyl group
Pr^i	isopropyl group
R_f	retention index for thin layer chromatography
TFA	trifluoroacetic acid
TFAA	trifluoroacetic anhydride
THF	tetrahydrofuran
tlc	thin layer chromatography
Tr	triphenylmethyl group
Ts	*para*-toluenesulphonyl group
V	phenoxyacetamido

INTRODUCTION

1.1 INTRODUCTION

1.1.1 History Of Penicillin

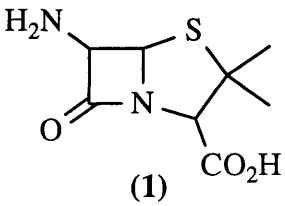
Preceding the romantic story of Fleming's observations, indeed as early as Biblical times, penicillin antibiotics in the form of hyssop were recommended in all cases of 'scall', 'plague', and 'leprosy'[1] which encompasses a whole range of superficial infections. The psalmist David beseeches[2] in the Old Testament, 'purge me with hyssop and I shall be clean.'. The relevance of these passages was clarified in 1911 following the first description[3] of *Penicillium notatum* and its isolation from a hyssop plant.

Several observations were documented[3] between 1870 and 1900 on the capacity of the fungi *Penicillium* to antagonise bacterial growth. Nevertheless they had no end result and whether any of them could be justified by penicillin activity will never be established.

Consequently[4] all that is perceived about the early history of penicillin dates from 1928, when Alexander Fleming observed incomplete lysis of staphlococci colonies on a plate that had been contaminated by a mould eventually identified as *Penicillium notatum.* Fleming cultivated his mould and appointed the name Penicillin to the active 'mould broth filtrate' he obtained. Furthermore he revealed that his crude penicillin filtrate had a potent but selective antibacterial activity and was no more harmful to the rabbit or mouse than ordinary broth. Within 10 years and after numerous successful clinical trials which proved penicillin was a powerful therapeutic agent in the fight against infection, the chemistry and structure of the molecule became the subject of a tremendous amount of research during and immediately following World War Π and on both sides of the Atlantic[5].

The first major advancement[6] occurred in 1958 when comparatively large amounts of the 'penicillin nucleus' (6-APA) **(1)** were formed using amidase

enzymes (isolated from the *Streptomyces* group) which react with penicillin V **(2)** and cleave off its side chain. This enzymatic cleavage of existing penicillins to



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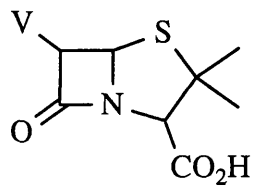
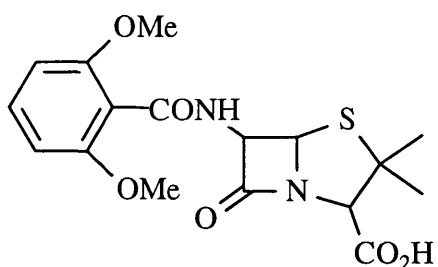


(2) V = $PhOCH_2CONH$

(phenoxyacetamido)

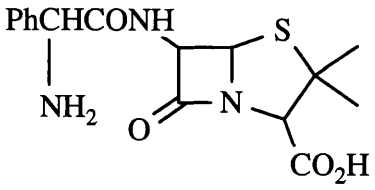
6-APA was first transferred to large scale production in 1959 and provided the opportunity for a systematic study of derivatives. Subsequent years saw a hectic search for semisynthetic penicillins manufactured by acylation of **(1)** and the results were numerous compounds that had considerable therapeutic advantages over naturally occurring penicillins.

Methicillin **(3)** was produced in 1960 by the Beecham Laboratories[7] from semi-synthesis from 6-APA and was shown to be resistant to staphylococcal penicillinase (an enzyme which destroys penicillins) and therefore effective against infections with penicillin resistant staphylococci.



(3)

A further milestone was the introduction[8] in 1961 of Ampicillin **(4)**, the first broad-spectrum penicillin, that was active against a whole range of bacterial strains. Unfortunately it was susceptible to penicillinases.



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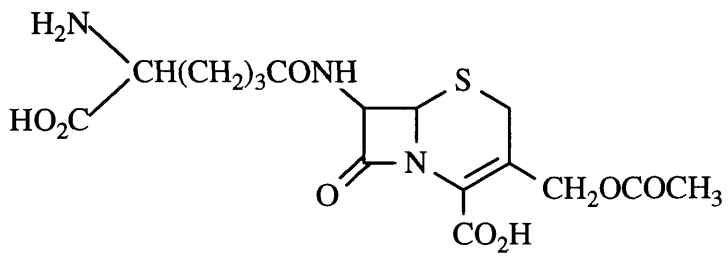
1.1.2 History of Cephalosporins.

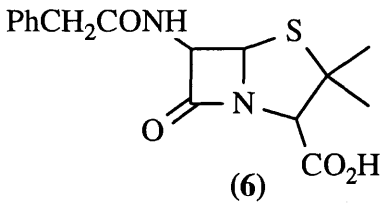
Subsequent to Florey, Chain, Heatley and Abraham's (Oxford group) demonstration of penicillin's chemotherapeutic properties, Guiseppe Brotzu[9] commenced a search for antibiotic-producing organisms in Sardinia. He was rewarded when he isolated a strain of *Cephalosporium acremonium* and discovered it produced antibacterial substances with broad spectrum activity. Further analysis of the active material by the Oxford team revealed that more than one antibiotic was present.

Cephalosporin C (**5**), the third antibiotic from the initial culture can be considered the origin of all true cephalosporins. Additional analysis showed that



(5)

some of its chemical and bacterial properties resembled those of penicillin with one exception - its resistance to hydrolysis by penicillinase - which proved instantaneously significant and highly desirable. Furthermore Howard Florey showed cephalosporin C had an even lower acute toxicity to mice than benzylpenicillin (**6**) which provided additional impetus for its continued study.



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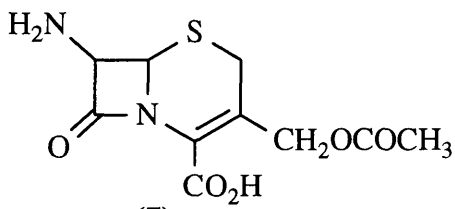
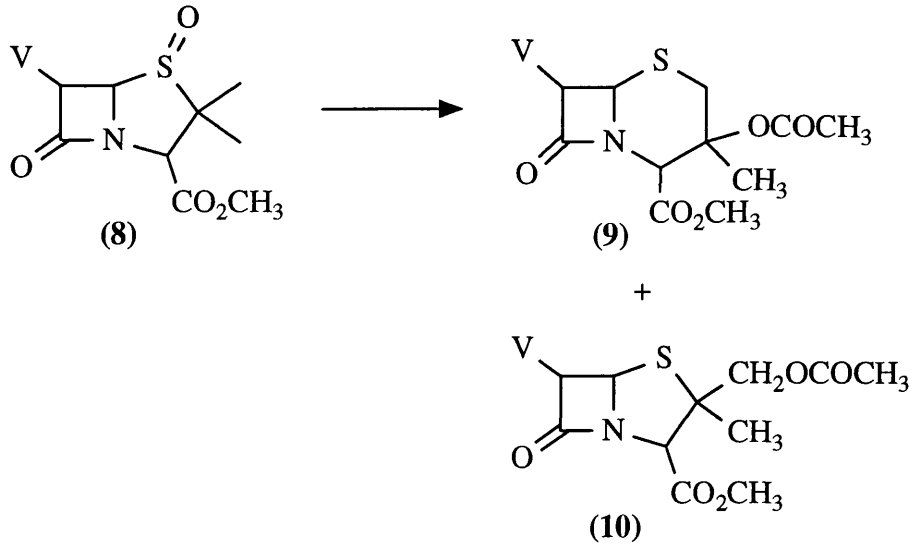
Approximately 10 years after its initial discovery, in 1959, the structure of cephalosporin C was elucidated by Abraham[10] as a β-lactam dihydrothiazine system. This structural assignment was later reinforced by X-ray crystallography[11]. Around the same time small amounts of the 'cephalosporin nucleus', (7-ACA), **(7)** were discovered through gentle acid hydrolysis of cephalosporin C.



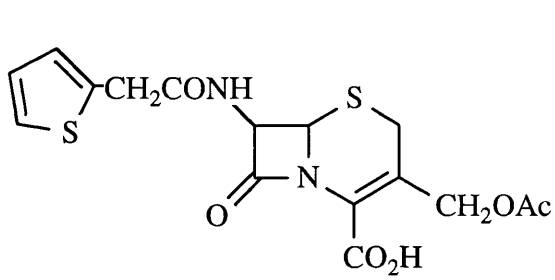
(7)

An intensive search began to find an enzyme (as had been accomplished with 6-APA) that would remove the α-amino adipyl side chain from cephalosporin C but these efforts proved fruitless and no active enzyme corresponding to the penicillin amidase has, to this day, been found. The cephalosporin project might have been abandoned had Morin and coworkers[12] not discovered a chemical preparation of 7-ACA in reasonable amounts. By refluxing methyl phenoxyacetamidopenicillinate sulphoxide **(8)** in acetic anhydride, they observed two products, a cephalosporin derivative **(9)** and a substituted penicillinate **(10)**.



Following this rearrangement of the penam system into the cephalosporin ring system, several reports discussed potentially better and easier ring expansion methods and, analogously to 6-aminopenicillanic acid, 7-ACA became the cornerstone for a rich source of derivatives that possessed higher antibacterial activity than their parent cephalosporins.

In 1964, 19 years after its initial discovery, two semi-synthetic cephalosporins were introduced into clinical use. The first produced by Eli Lilly & Company[13] was Cephalothin **(11)**, containing a thienylacetyl side chain. The second produced by Glaxo Laboratories[14], was Cephaloridine **(12)**, which incorporated a pyridinium cation instead of Cephalothin's O-acetyl group.



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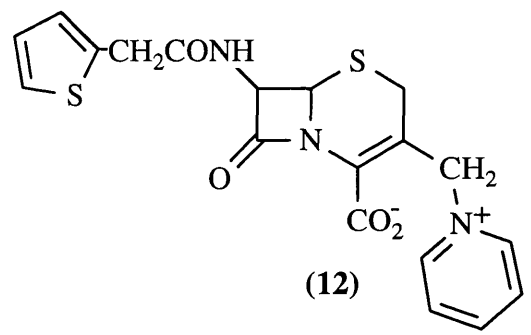
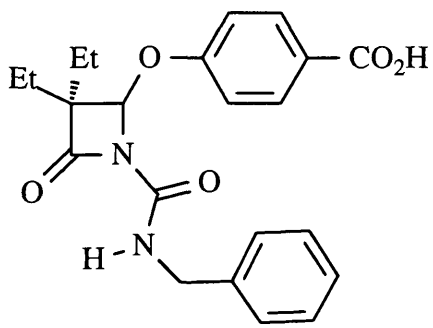


(12)

Today penicillin and cephalosporin research is less diligent than before but the area has been expanding with increasing momentum leading to the

introduction of new β-lactams with properties that are potentially beneficial eg **(13)**. These new compounds are neither penicillin nor cephalosporin based and essentially their general attribute is the β-lactam ring. Over the last 10 years more than 50, 000 novel compounds have been synthesised and tested and consequently despite 60 years of study the β-lactam antibiotics continue to bewilder, intrigue and frustrate scientists.



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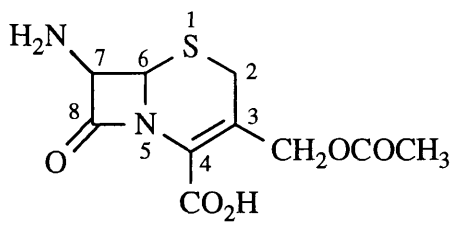
Although this project is concerned with positions C-2 and C-4 of the ceph-3-em nucleus **(7)**, the following sections detail research accomplished at the various other positions and the subsequent effect they have on bacterial activity.



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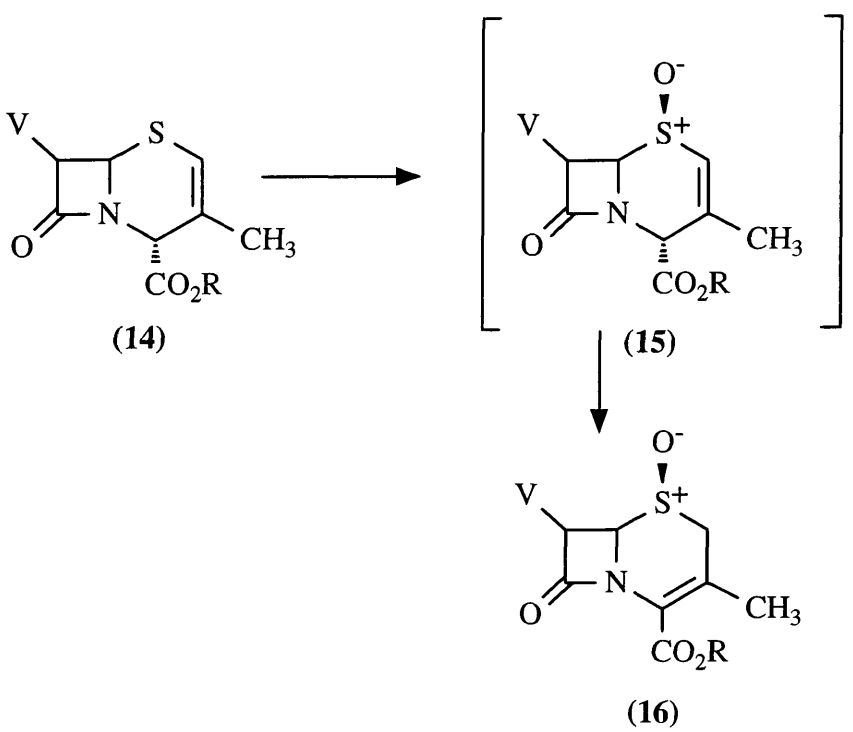
1.2 Reactions at Sulphur

1.2.1 Oxidation

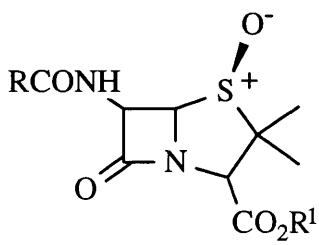
Although oxidation tends to lead to less biologically active compounds they are nevertheless of great synthetic value. Uses range from functionalisation of the cephalosporin C-2[15] (see section 1.3); conversion of ceph-2-ems to

ceph-3-ems[16]; allylic halogenation at the C-3 of deacetoxycephalosporins[17&18] and the penicillin sulphoxide acts as an intermediate in the acid-catalysed ring expansion to cephalosporins[19]. Furthermore cephalosporin sulphones are reported to be powerful inhibitors of human leucocyte elastase[20].

As a consequence of the conformation of the dihydrothiazine ring, oxidation would be expected to occur on the sterically less hindered α-face. However it has been reported[21] that cephalosporins (and penicillins) incorporating a C-7 (C-6) amide proton result in the formation of the β-sulphoxide, with readily available oxidants eg *m*-CPBA or peracetic acid. The recognised explanation for this preference is the reagent approach control theory ie hydrogen bonding occurs between the proton of the β-amide moiety at the C-6 or C-7 position and the oxidant directing oxidation from the more hindered β-face. An important publication, by Kaiser and colleagues[22] was on the application of a variety of percarboxylic acids to convert Δ2-cephem esters **(14)** via the intermediate **(15)** into the more thermodynamically stable Δ3-cephem sulphoxides **(16)**.



Employing *m*-CPBA or periodic acid gave **(16)** in high yields (70-90%) whereas weaker oxidising agents such as hydrogen peroxide and peracetic acid afforded **(16)** in slightly lower yields with longer reaction times. It has also been noted[22&23] that use of hydrogen peroxide with a large excess of organic acid tended to cleave the β-lactam moeity with poor yields of sulphoxide, however yields of 85-94% were acheived[24] when the penicillin and cephalosporin sulphoxides **(17)** and **(18)** were prepared with hydrogen peroxide in dichloromethane using three or four molar equivalents of formic or acetic acid.



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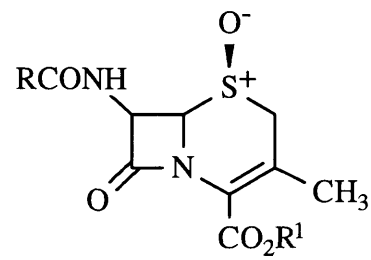
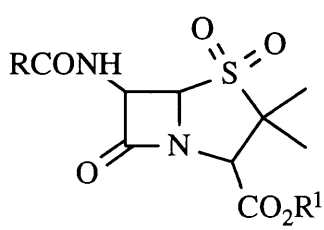
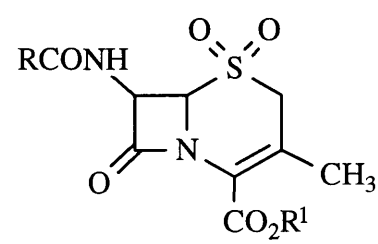


(18)

Furthermore this reagent mixture reduced acidic decomposition, reaction times and minimised formation of the sulphone. These conditions were further researched by Micetech *et al*[25] who reported that oxidation with H_2O_2 in dichloromethane and acetic acid gave only the sulphoxides **(17)** and **(18)**. However in the presence of formic acid, oxidation occurred twice and the sulphones **(19)** and **(20)** were obtained in inseparable mixtures. Increasing the reaction times and amount of formic acid used afforded high yields (72-90%) of **(19)** and **(20)**.

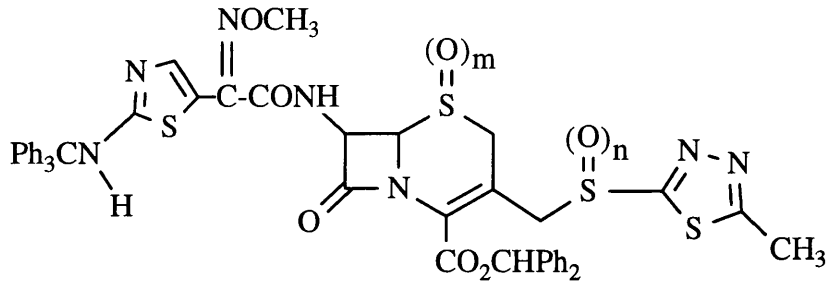


(19)



(20)

Singh and his Canadian co-workers[26]considered the oxidation of ceph-3-em **(21)** containing two thioether functions ie the dihydrothiazine ring sulphur and the 3-CH_2-S, both of which are susceptible to oxidation.



(21) m = n = 0

(22) m = 1, n = 0

Utilising both *m*-CPBA and H_2O_2, cephalosporin **(22)** was produced in 63-80% yields. This ceph-3-em displayed a potent activity against gram negative organisms but only moderate activity with gram positive bacteria.

With reference to the ability of the C-6 and C-7 amide group of penicillins and cephalosporins to direct the oxidation from the β-face via steric approach control, the problem of the α-sulphoxide production was overcome by removal of this directive effect resulting in predominant formation of the penicillin and cephalosporin α-sulphoxide by steric factors. Three possible methods were used and involve:-

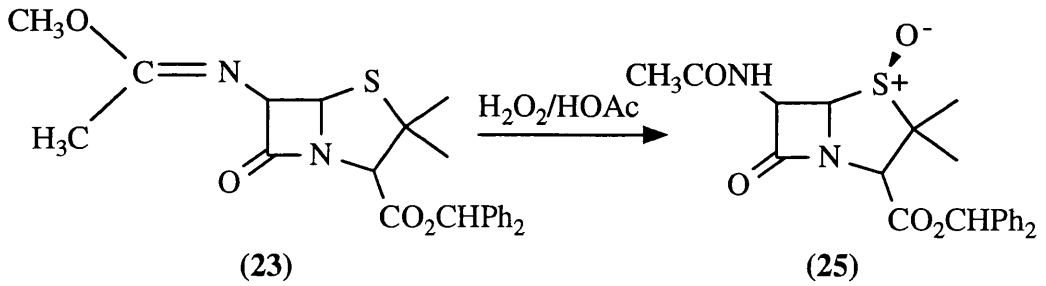
a) protection of the C-6 or C-7 amide proton eg formation of N-chloroimines[27],

b) use of an oxidant that will not partake in hydrogen bonding eg oxone[28],

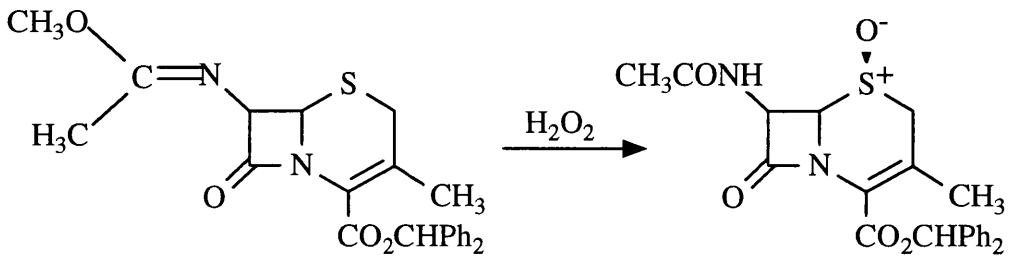
c) reagents such as N-monochlorourethane[29] and N,N-dichlorourethane[30] which establish a β-sulphonium chloride first and then invert their configuration on hydrolysis.

Using a protective route, Micetich and his Canadian coworkers[31] attempted the oxidation of penicillin **(23)** and cephalosporin **(24)** with H_2O_2 in the presence of acetic acid. However, they reported the formation of the β-sulphoxides

(25) and **(26)** instead of the corresponding α-sulphoxides. These results were explained by initial addition of the oxidising agent to the iminoether, thus facilitating oxidation at the hindered β-face.

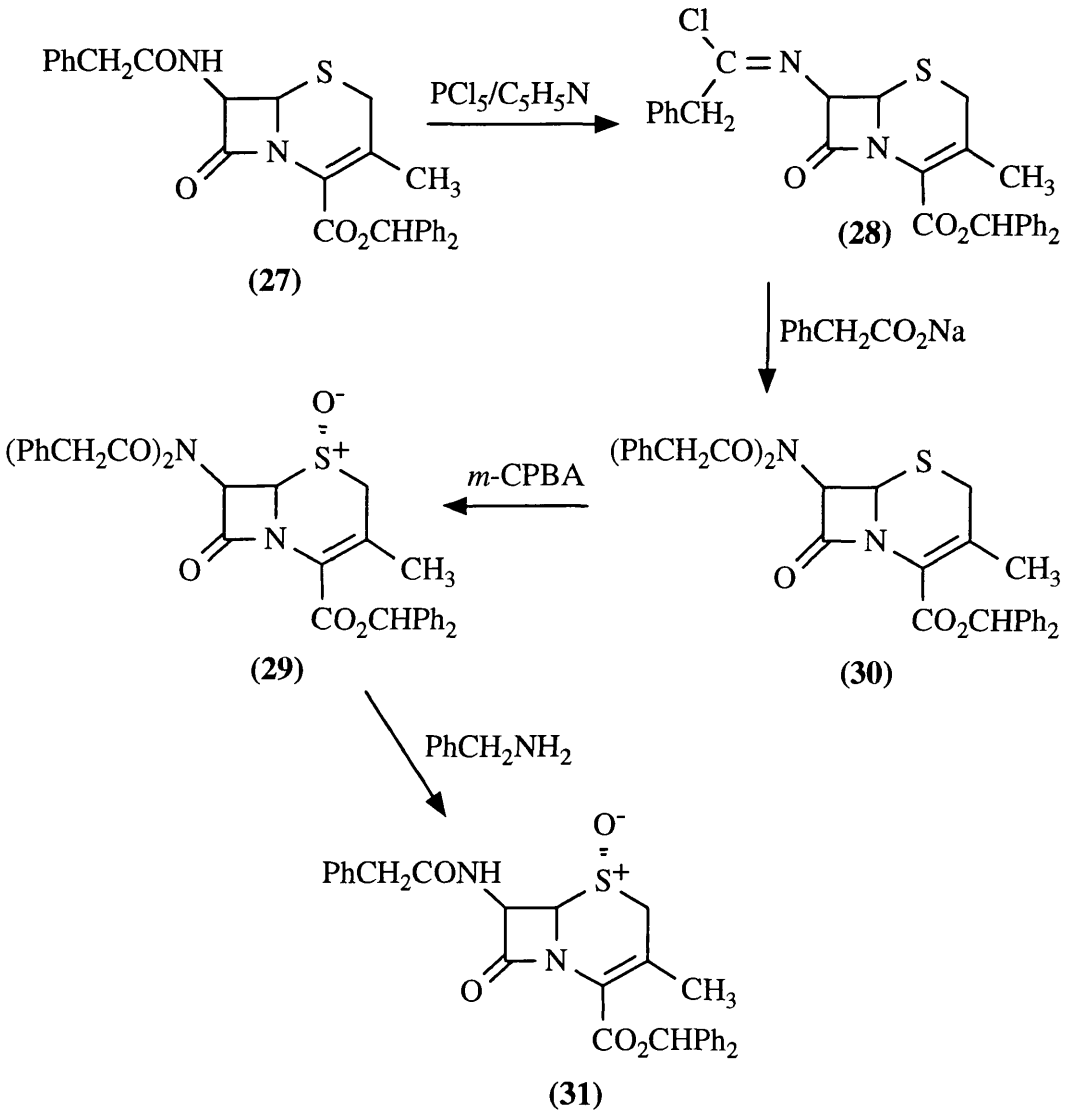


(23) **(25)**

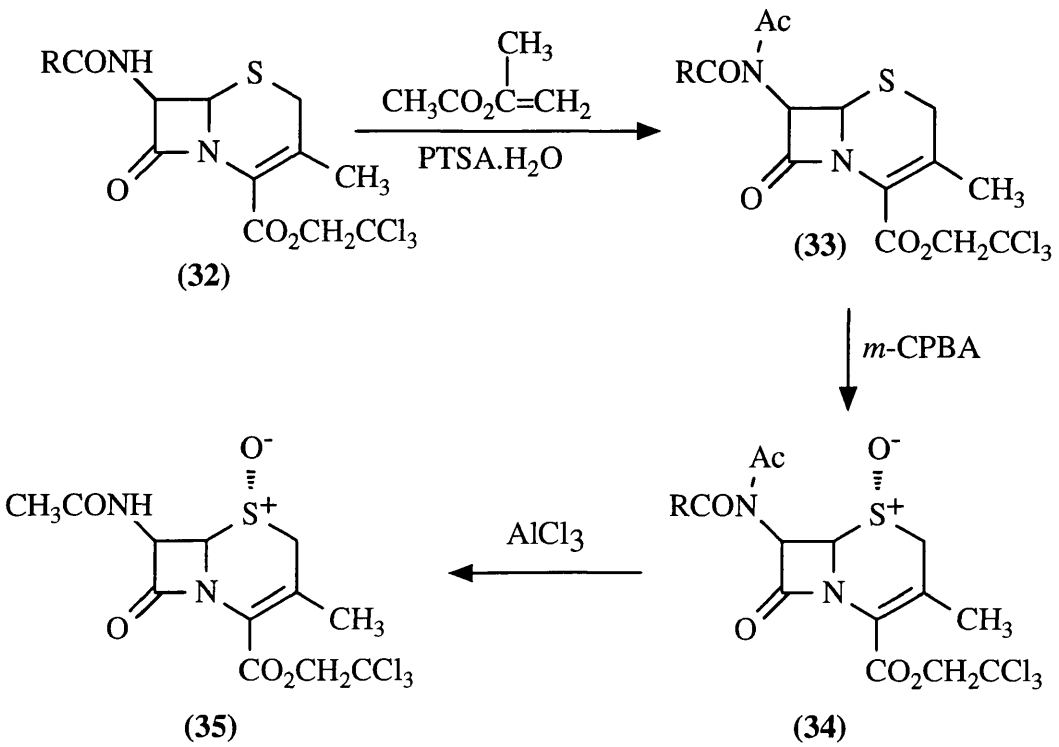


(24) **(26)**

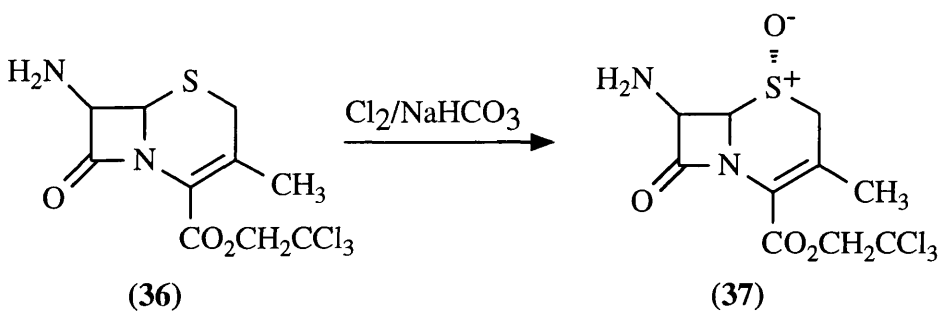
Furthering their research they reported[32] a simple procedure of converting the 7-amidoceph-3-em **(27)** with phosphorus pentachloride in benzene into the 7-chloroimine **(28)** which readily reacted with an acid salt to afford the 7-diacylamino-ceph-3-em **(29)** in 50.4% yield. Oxidation with *m*-CPBA resulted in a 95% yield of the 7-diacylaminoceph-3-em 1α-oxide **(30)** which was subsequently deprotected with benzylamine in benzene or toluene to afford the 7-phenylacetamidoceph-3-em 1α-oxide **(31)** in 37% yield.



Additionally, Spry *et al*[33] have investigated the possible uses of C-7 imides in the synthesis of α-sulphoxides. Ceph-3-em (**32**) reacted with isopropenyl acetate and *p*-toluenesulphonic acid affording (**33**) which on oxidation with *m*-CPBA afforded the α-sulphoxide (**34**). Deprotection using $AlCl_3$ gave a high yield of the 7-amidoceph-3-em 1α-oxide (**35**).



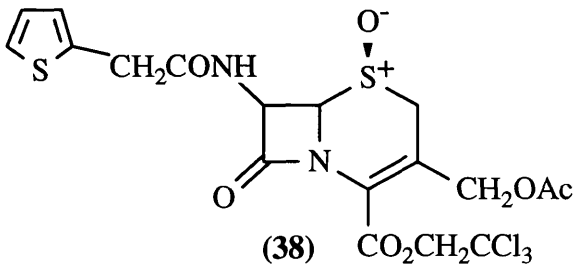
Furthermore, they reported[34] a straightforward oxidation of ceph-3-em **(36)** using Cl_2 and $NaHCO_3$ affording the 1α-sulphoxide **(37)** in reasonable yields.



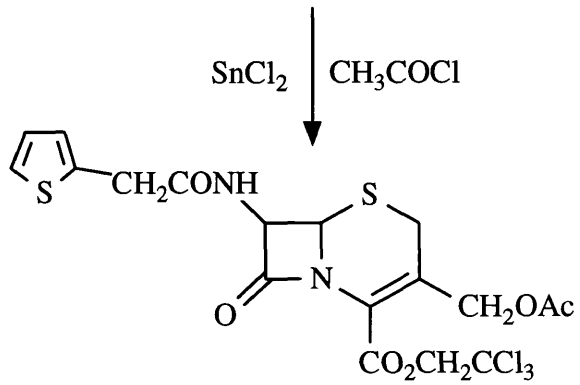
1.2.2 De-oxygenation

An early attempt by Cocker and colleagues[16] to reduce Δ3-cephem sulphoxides using a wide variety of conventional reducing agents proved fruitless. It was suggested[21] that electronic factors were the cause of this resistance to reduction as there appeared to be no steric inhibition of the reducing agent itself. Effectively, the sulphur-oxygen bond is strengthened as a result of the

electron-withdrawing effect of both the β-lactam nitrogen and the α, β-unsaturated carboxyl function. To overcome this, the sulphoxide needs to be activated by a reactive acid halide and it can then be de-oxygenated with a number of common reducing agents. For example ceph-3-em **(38)** dissolved in CH_3CN and DMF is

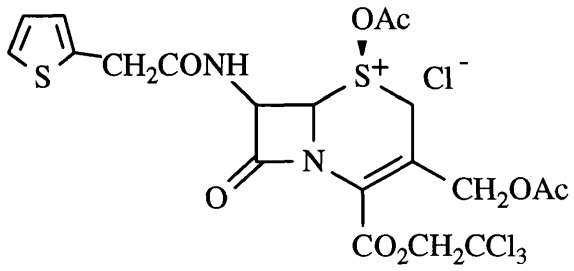


(38)



(39)

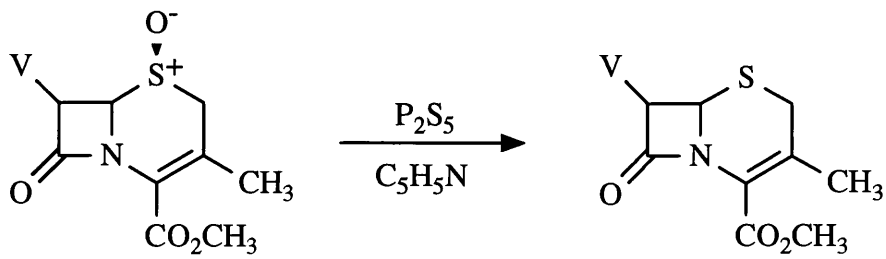
de-oxygenated after 1 hr at 0°C in the presence of stannous chloride and acetyl chloride affording sulphide **(39)** in 98% yield. It was proposed that acetyl chloride reacts with the sulphoxide oxygen forming the sulphoxonium salt **(40)** which is more reactive towards the reducing agent. In addition several reagents such as



(40)

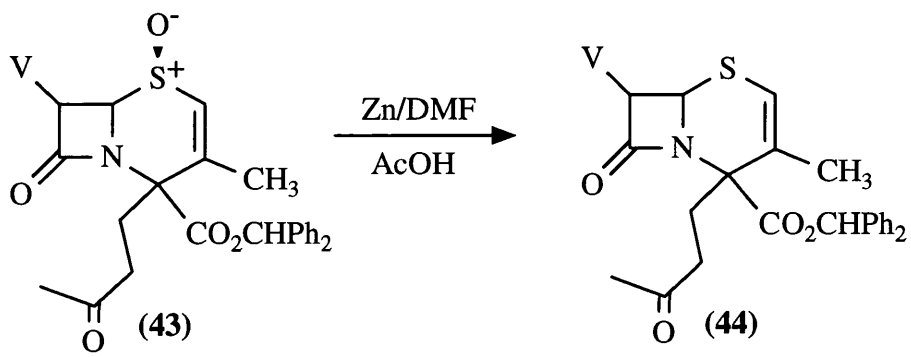
PCl_3, PBr_3 and $SiHBr_3$ possess acid halide character and complete de-oxygenation occurs without the presence of an activating agent. Micetech[35] has subsequently

discovered that P_2S_5 in the presence of pyridine is an effective reagent for the de-oxygenation of both penicillin and cephalosporin sulphoxide analogues eg **(41)** affording the sulphides **(42)** in high yields (90%). Under normal reducing



(41) **(42)**

conditions Δ3-cephalosporins are not de-oxygenated, however, it appears Δ2-cephems eg **(43)** succumb[36] to zinc in DMF containing glacial acetic acid resulting in the sulphide **(44)** suggesting no such electronic effects exist in these molecules.

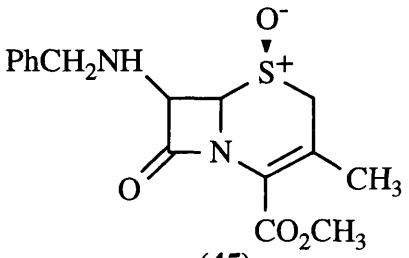


(43) **(44)**

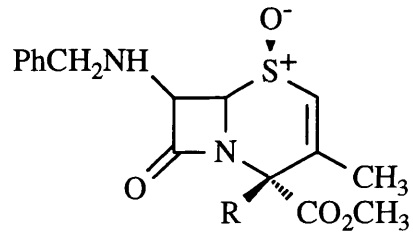
1.3 Reactions at C-2

1.3.1 2-Alkylcephems

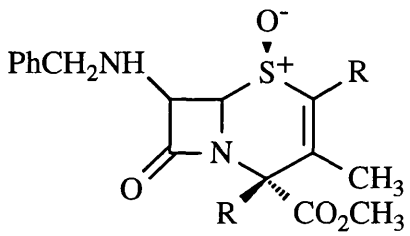
Few publications exist concerning cephalosporins prepared by simple alkylations, however Yoshida *et al* have described[37] the methylation of the sulphoxide **(45)** using methyl iodide and 1 mole equivalent of sodium hydride. The products included the 4β-methylceph-2-em sulphoxide **(46a)** in a yield of 74% and the di-adduct 2,4-β-dimethylceph-2-em **(47a)** in a 9.5% yield arising from initial formation of the 2α-methyl ceph-3-em **(48)**.



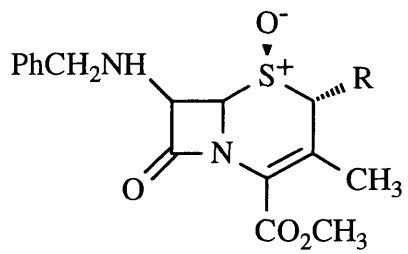
(45)



(46)



(47)

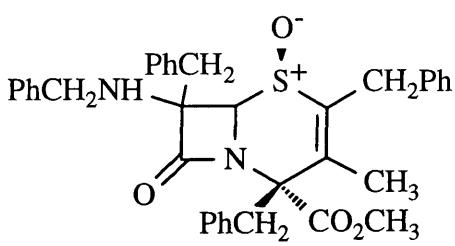


(48)

a; R = Me

b; R = CH_2Ph

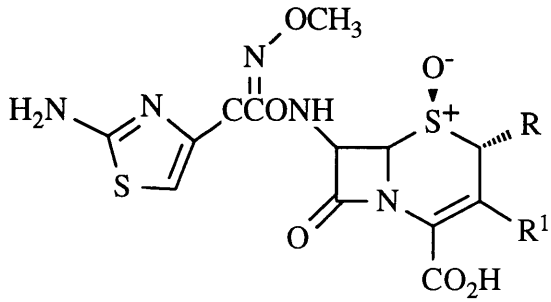
Similarly benzylation of (**45**) was carried out to afford (**46b**) and (**48b**) in 25% and 27% yield respectively. Di-adduct (**47b**) was also isolated in a 25% yield. Furthermore, using 3 mole equivalents of lithium diisopropylamide instead of sodium hydride, resulted in the dibenzylated sulphoxide (**48b**) in 10% yield and the tribenzylated derivative (**49**) in a yield of 12%.



(49)

Takaya and coworkers[38] investigated the *in vitro* microbiological activity of the 2- and/or 3-methylcephalosporins (**50**). No details were given for the preparation of these compounds. The presence of a 2α-methyl substituent incorporated with a 3-methyl group as displayed by cephalosporin (**50c**) resulted in a significant reduction of biological activity. An effective improvement was achieved by removing the 3-methyl group as in (**50a**). However converting (**50a**)

to its stereochemical isomer, ie 2β-methyl enantiomer (**50b**) reduced biological activity in comparison. Further enhancement was observed when neither C-2 nor C-3 methyl groups were present as shown in (**50e**).

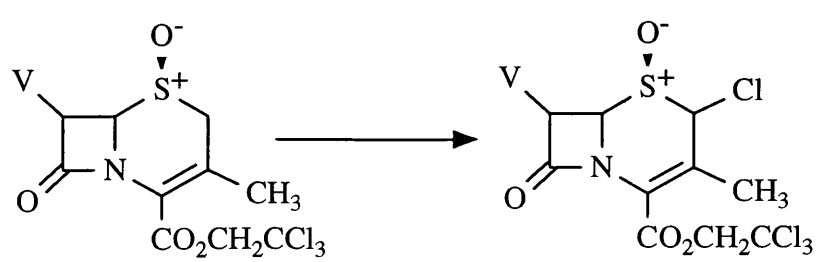


		R	R^1
(**50**)	a;	α-CH_3	H
	b;	β-CH_3	H
	c;	α-CH_3	CH_3
	d;	H	CH_3
	e;	H	H

1.3.2 2-Alkoxycephems

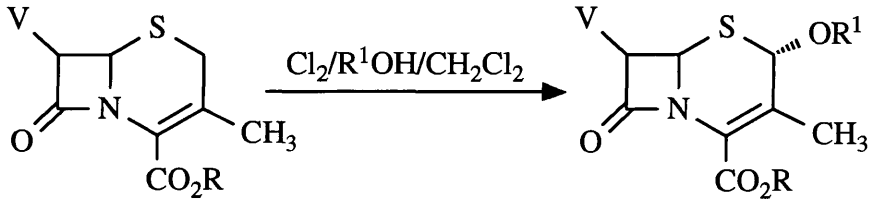
A wide variety of C-2 substituted cephems have been synthesised by replacement of either methoxy or acetoxy substituents and hence interest has continued in developing more facile preparations of these compounds.

In 1972 Spry[39] investigated the possibility of using α-chloro sulphoxides as precursors to C-2 substitued cephalosporins. Treatment of (**51**) with, either, sulphuryl chloride or chlorine in pyridine/dichloromethane mixture resulted in the α-chloro sulphoxide (**52**). The sulphide (**53**) also undergoes chlorination to a



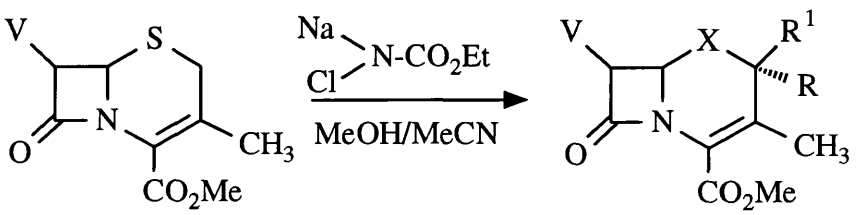
(**51**) (**52**)

α-chloro adduct which was trapped with various alcohols to form 2-alkoxy ceph-3-ems (**54**). The corresponding 2-alkoxy cephalosporanic acids (**54; R=H**)



(**53**) (**54**)

all displayed antibacterial activity. Further reactions involving (**52**) failed confirming Leoppky and Chang's results[40] that α-chloro sulphoxides are unreactive with nucleophiles. Campbell, Bremner and Johnston[41] discovered that reaction of sulphide (**42**) with N-chloro-N-sodiourethane in a mixture of methanol-acetonitrile resulted in a 20% yield of the 2-methoxycephem (**55**). In a similar reaction the corresponding sulphoxide (**41**) gave the dichlorocephem (**56**).

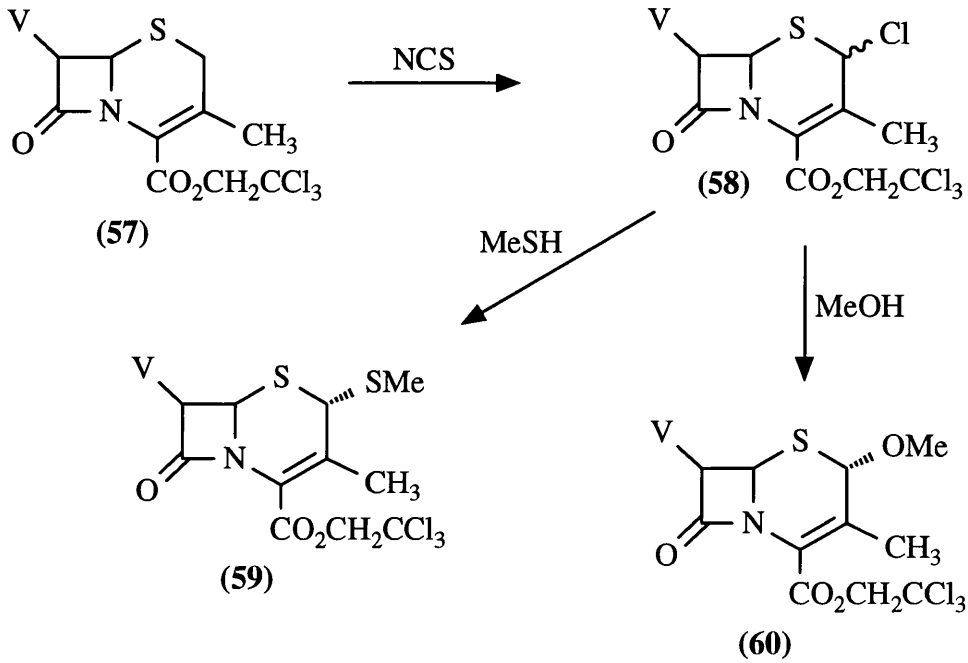


(**42**)

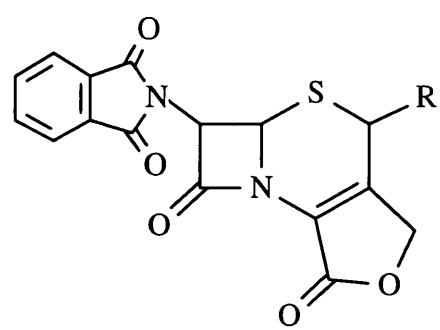
(**55**) X = S, R = OMe, R^1 = H

(**56**) X = S-O, R = R^1 = Cl

A separate publication reported[42] that reaction of (**57**) with N-chlorosuccinimide (NCS) resulted in the unstable 2-chloroceph-3-em (**58**) (observed in solution by nmr). Nucleophilic displacement at C-2 in (**58**) was induced by treatment with methanethiol or methanol resulting in the 2α-methylthio and 2α-methoxy cephems, (**59**) and (**60**) respectively. In an earlier paper[43] by the



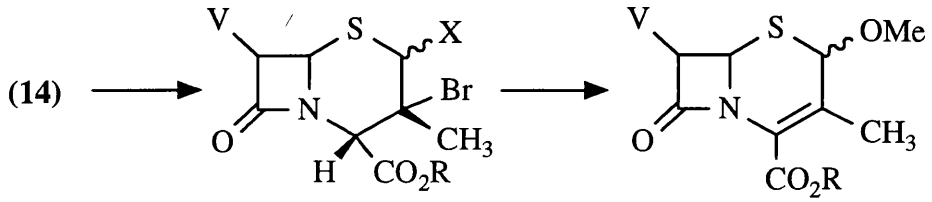
same authors bromination of **(61a)** was described with N-bromosuccinimide affording **(61b)** which is converted to its corresponding 2-methoxy adduct **(61c)** *via* the addition of triethylamine to a methanolic solution of **(61a)**.



(61) a; R = H
b; R = Br
c; R = O Me

Macchia and co-workers report[44] the addition of bromine to Δ2-cephalosporins **(14)** which furnished either the 2,3-dibromo- or 3-bromo-2-methoxy cepham products **(62)** and **(63)** depending on solvent conditions used. Treatment with a dilute solution of bromine in either carbon tetrachloride or dichloromethane results in the dibromo adduct **(62)** but using

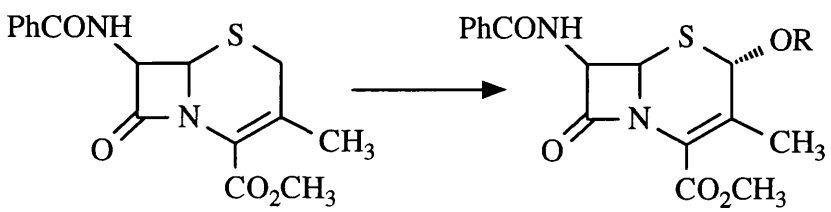
methanol as the solvent, the 2-methoxycepham **(62b)** is obtained. Dehydrogenation of **(62b)** with triethylamine in benzene at room temperature resulted in a mixture of α and β-isomers of ceph-3-em **(63)**. Both 2α-methoxy and 2β-methoxycephalosporanic acids showed similiar antibacterial activity against gram positive bacteria only.



(62) a; X = Br
b; X = OMe

(63)

A direct preparation[45] of C2-alkoxycephems **(65)** from the 7-benzylamido ester **(64)** was accomplished with *tert*-butylhypochlorite in alcohol and, more recently, direct alkoxylation has been acheived[46] by electrolysis at 1.1V in methanol/THF (3:1) using tetraethylammonium tosylate furnishing the 2-methoxy derivatives **(63; R = PNB, DPM & Bu^t)**. Altering the solvent to

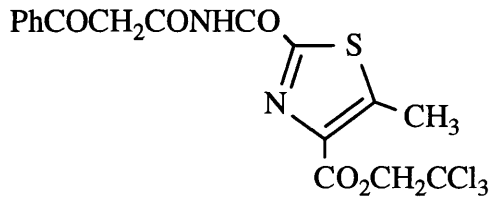


(64)

(65) R = Me
R = Et

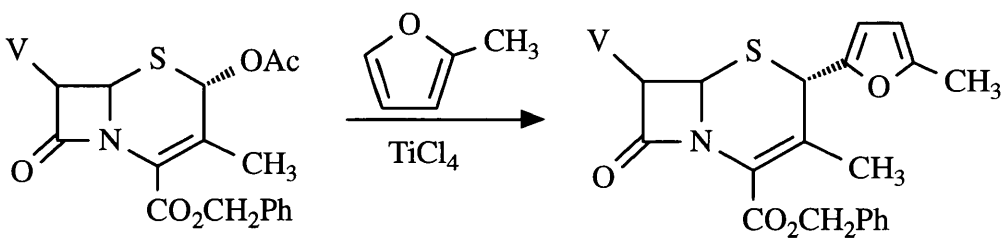
ethanol, propan-2-ol, or benzyl alcohol resulted in the 2-ethoxy, isopropoxy and benzyloxy derivatives respectively in yields of 40-70% depending on the cephalosporin ester group. Similiarly[47], treatment of **(57)** with cerium (IV) ammonium nitrate (CAN) in the presence of methanolic THF afforded the 2-methoxycephem **(60)** in 50%. Interestingly, an earlier publication[48] by the same authors reported reaction of **(57)** in the presence of CAN under acidic conditions

resulted in degradation of the β-lactam ring to give the crystalline thiazole product **(66)**.



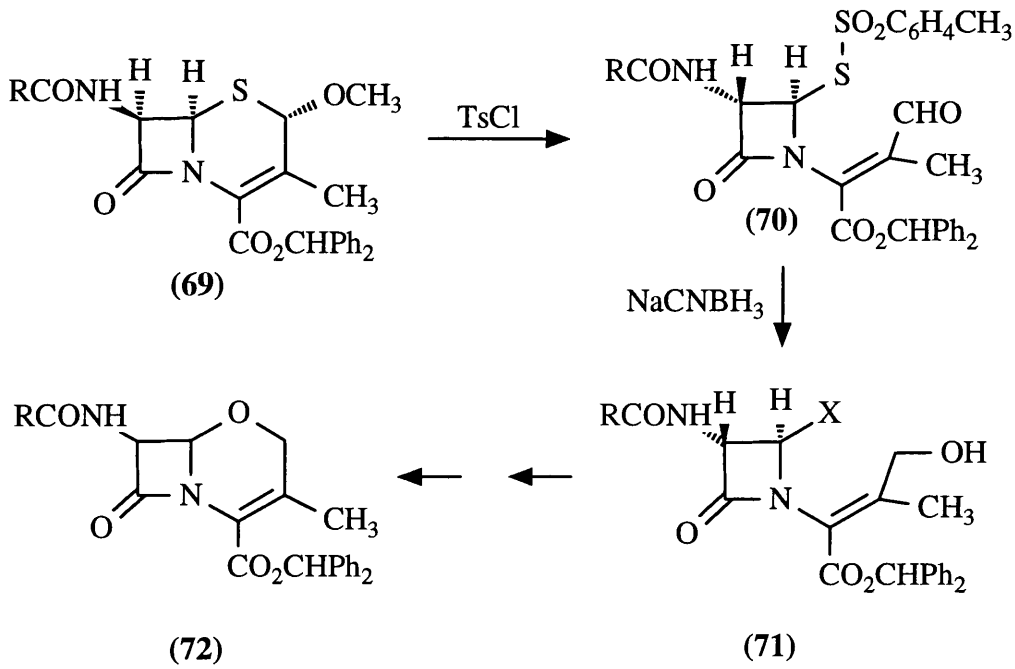
(66)

Utilizing an unconventional method, Torii *et al*[49] synthesized a wide range of more complex C-2 substituted cephems from 2-alkoxycephems. A C-2 cation was generated *via* reaction of either 2-acetoxy or 2-methoxy cephalosporin with a Lewis acid catalyst (eg: $TiCl_4$, $AlCl_3$) and further reacted with a nucleophile. Hence, treatment of **(67)** with 2-methylfuran in the presence of titanium (IV) chloride at -25°C for 25 min afforded the 2-furylcephem **(68)** in 87% yield. Various nucleophiles were added in this manner, including furan, 2-methylthiophene, N-methylpyrrole, allyl alcohol and butanethiol, affording the desired C-2 substituted cephems in yields between 55-96%.



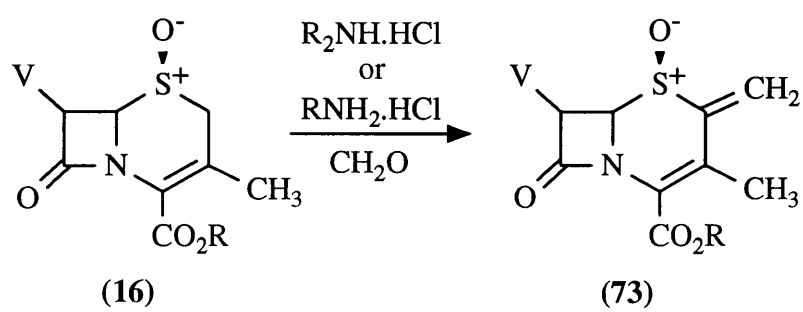
(67) **(68)**

In addition, 2-methoxycephems have been used in the preparation of 2-oxacephems[50]. The dihydrothiazine ring of **(69)** is cleaved in the presence of tosyl chloride furnishing the disulphide **(70)** which is reduced on treatment with sodium cyanoborohydride to the alcohol **(71)**. Mercuric chloride induces ring closure and C-7 epimerisation is accomplished with LiOMe and *t*-butyl hypochlorite to give 2-oxacephem **(72)**.

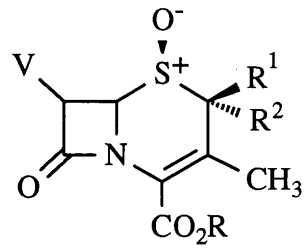


1.3.3 2-Exocyclic-Cephems

The introduction of exocyclic double bonds at the 2-position was initially discovered[51] when various ceph-3-em sulphoxides (**16**) were reacted with formaldehyde and a variety of primary and secondary amine salts under Mannich conditions to give 2-exomethylene derivatives (**73**) in 81-95% yield. Also reported

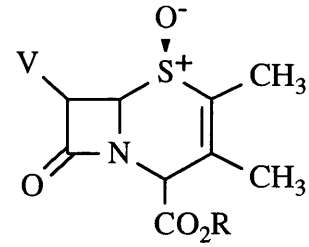


is the reaction of (**73**) with hydrogen over Pd/Rh catalysts to give (**74a**), (**74b**) and (**75**); treatment of (**73**) with a variety of thiols (R^3SH) to give (**76**) and finally rapid addition of bromine to afford cephem (**77**). De-oxygenation and de-esterification



(74) a; $R^1 = CH_3$, $R^2 = H$

b; $R^1 = H$, $R^2 = CH_3$



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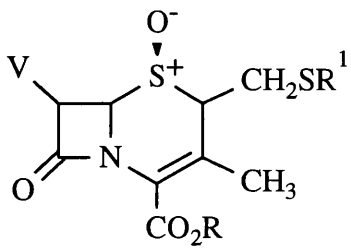

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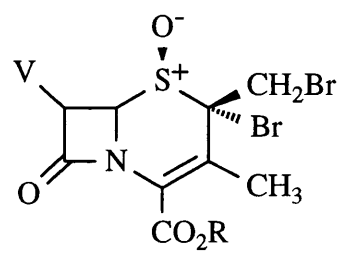
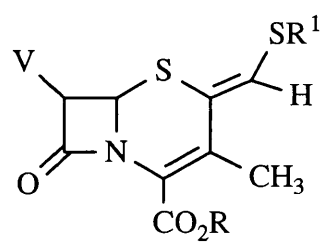


(77)

of these novel 2-substituted cephalosporins followed resulting in the corresponding cephalosporanic acids with increased anti-bacterial activity over the corresponding acid of **(16)**. In later work[52], the same group discovered that not only alkylthiols but a variety of aryl, alkaryl and heterocyclic thiols add to the 2-exomethylene position furnishing sulphides **(76)** in high yields. Furthermore, although generally stable, in the presence of acetic acid, products **(76)** lose water to form the cephems **(78)** as single Z-isomers.

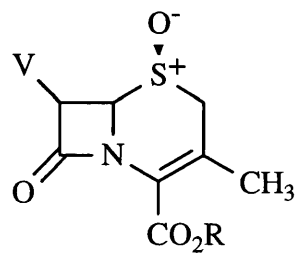


(78)

R^1 = alkyl, aryl, alkaryl or heterocyclic

After reduction the new cephalosporanic acids exhibited biological activity against penicillin-resistant *Staphylococcus aureus* but no significant gram-negative activity was observed.

Confirming these results, Jaszberenyi and coworkers[53] produced 2-exomethylene β-sulphoxides (**73**) and analogous sulphones, (via the Mannich reaction) from their corresponding cephems (**16**). However under similiar



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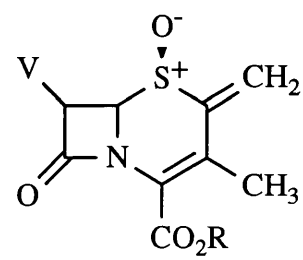
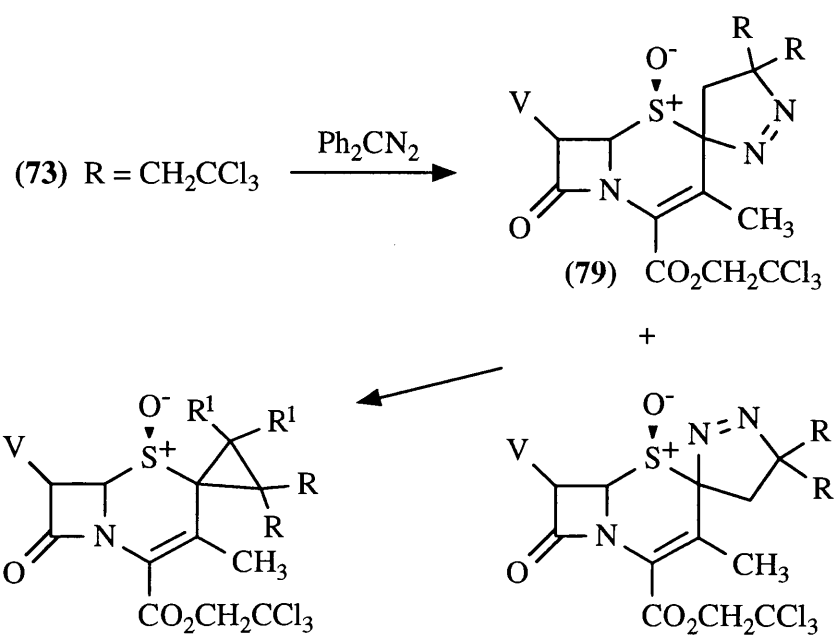


(73)

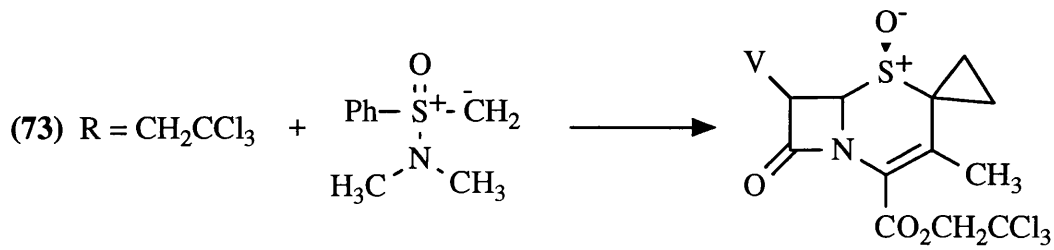
conditions the analogous α-sulphoxides were unreactive. A later publication[54], by the same authors, describes how yields of (**73**) produced under Mannich conditions were improved to 88% using dimethylamine hydrochloride, 36% aqueous formaldehyde solution, butanol and dichloromethane. A 1,3-dipolar cycloaddition of (**73; R=CH_2CCl_3**) was accomplished in the presence of diphenyldiazomethane at -15°C presumably to form (**79**) and (**80**) which spontaneously eliminate nitrogen to give (**81a**) in 86% yield and (**81b**) as a minor product.



(81) a; R = H, R^1 = Ph
b; R = Ph, R^1 = H

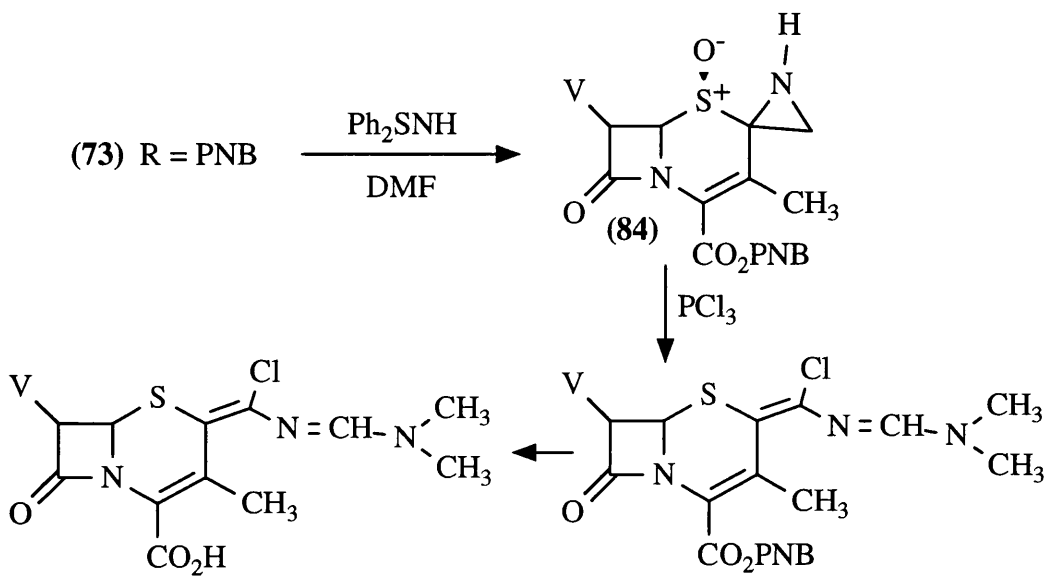
(80)

D Spry[55] utilises the 2-exomethylene cephem (**73; R=CH_2CCl_3**) along with the sulphoxonium ylide (**82**) in the preparation of 2-spirocyclopropyl cephalosporins (**83**). Other 2-cyclocephems (**84**) were synthesised from (**73;**



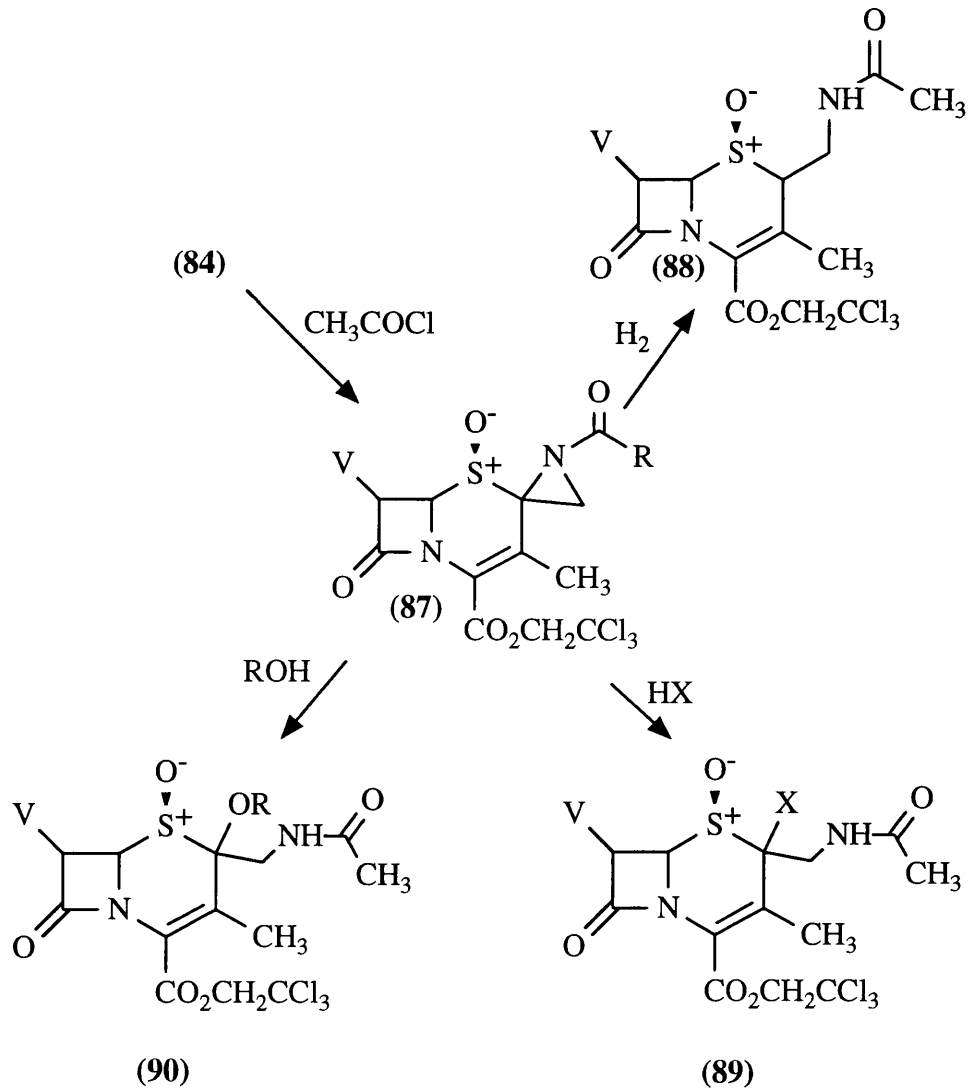
(**82**) (**83**)

R=PNB) using diphenylsulphilimine in DMF and attempts to de-oxygenate with PCl_3 in DMF resulted in the chloramidine (**85**) in a yield of 37%. Ester cleavage afforded the corresponding acid (**86**) which displayed less biological activity when compared to the acid of the 2-methylene derivative (**73**).

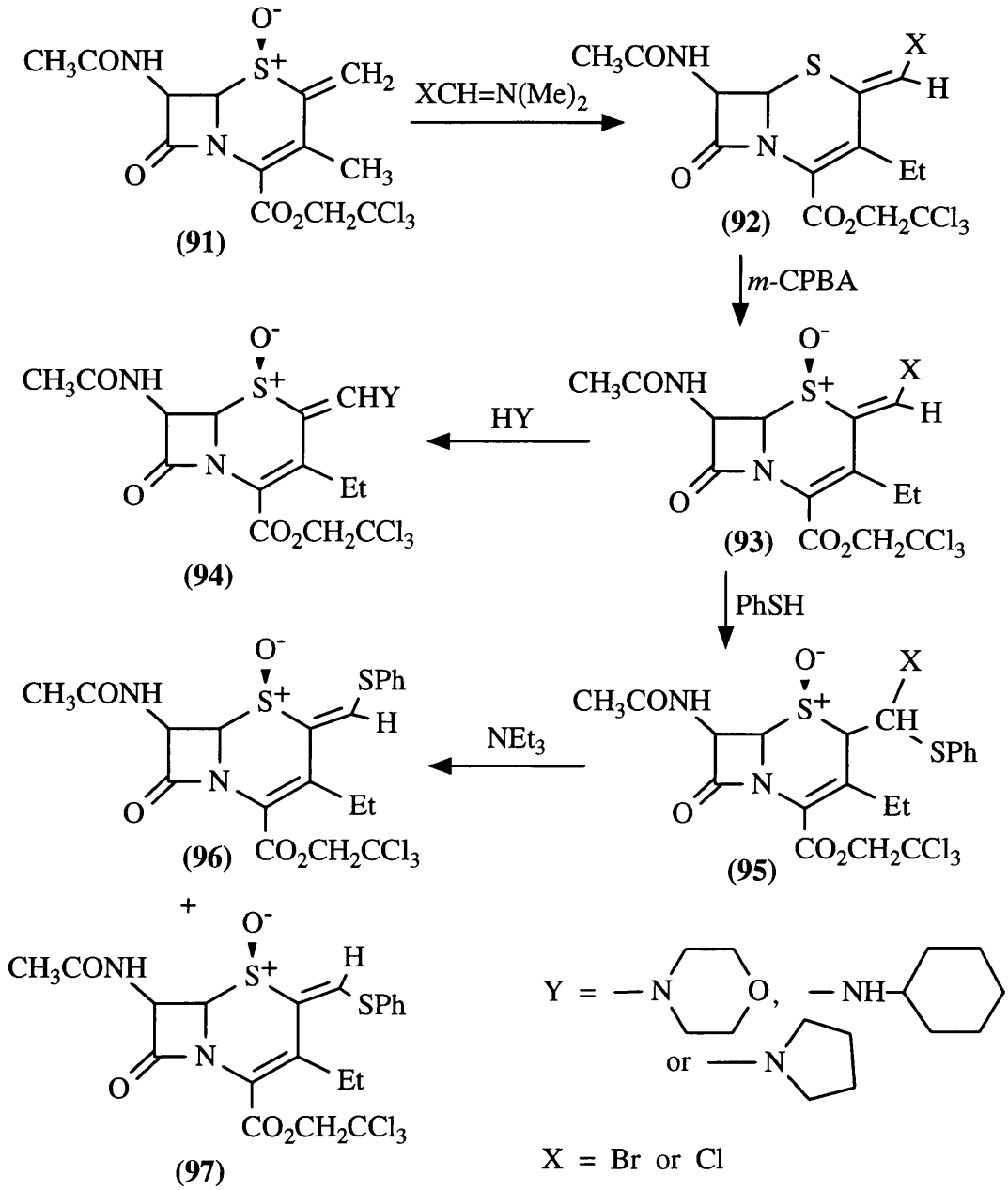


(**86**) (**85**)

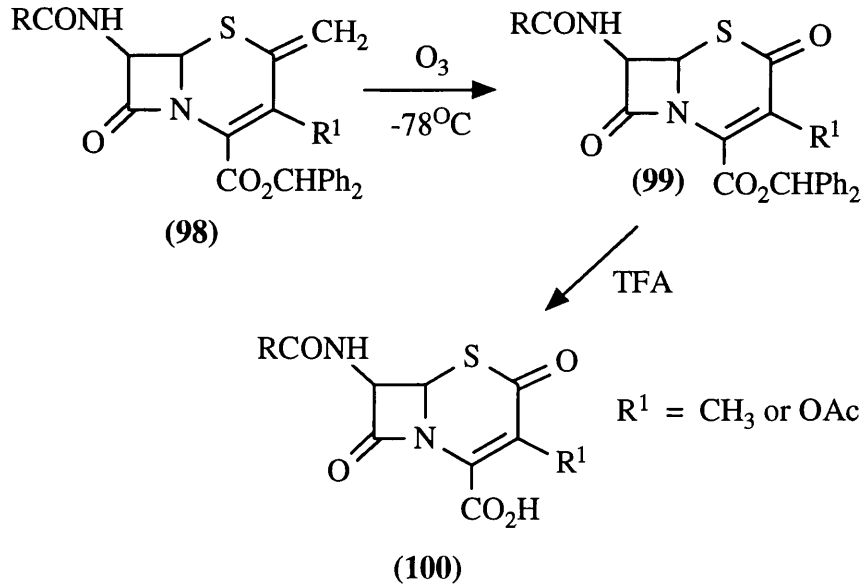
Spry[56] also acylated (**84**) using CH_3COCl to give the 2-spiroacylaziridine (**87**) which was further reacted with hydrogen, HX or various alcohols to furnish the amide (**88**), 2-halo-2-acylaminomethyl derivatives (**89**), or the 2-alkoxy-2-acylaminomethyl derivatives (**90**) respectively. Again the analogous acids all showed reduced activity in comparison to the acid of (**73**).



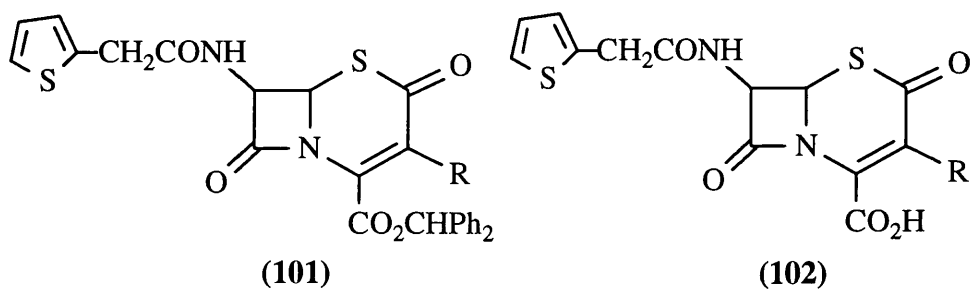
In a later publication[57], the reaction of **(91)** with dimethylimmonium halides in a Pummerer type rearrangement was investigated. The 2-methylene compound **(91)** was reacted with Vilsmeier reagent (PX_3 in DMF) affording product **(92)** in 50-82% yield. Oxidation with *m*-chloroperoxybenzoic acid formed **(93)** which on treatment with various primary and secondary amines produced crystalline enamines **(94)** in yields of over 57%. The vinylhalo sulphoxide **(93)** was also reacted with thiophenol to afford **(95)** which, in the presence of triethylamine, readily formed products **(96)** and **(97)** in 34% and 19% yields respectively.



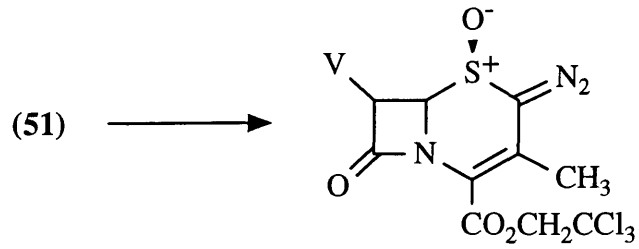
2-Oxocephalosporins are another range of ceph-3-ems with a double bond exocyclic to the dyhydrothiazines ring at the 2-position. Kim, Misco and McGregor[58] predicted that these compounds with the crucial Δ3 bond in conjugation with the 2-keto group would modify the reactivity of the β-lactam carbonyl and thereby increase biological activity. Preparation was carried out by briefly exposing the 2-methylenecephalosporins **(98)** to ozone at -78°C to furnish **(99)** followed by de-esterification with TFA and anisole to produce **(100)**.



Cleavage of the phenoxyacetyl group using PCl_5 and *p*-TsOH of (**99; R=PhOCH$_2$**) followed by reaction with 2-thienyl acetyl chloride gives (**101**). Ester cleavage resulted in the ceph-3-em acid (**102**). Neither (**100; R=CH$_3$**) or (**102; R=CH$_3$**) displayed any significant activity, however the 3-acetoxymethyl substituent of (**102**) resulted in enhanced activity to its corresponding 3-methyl derivative.

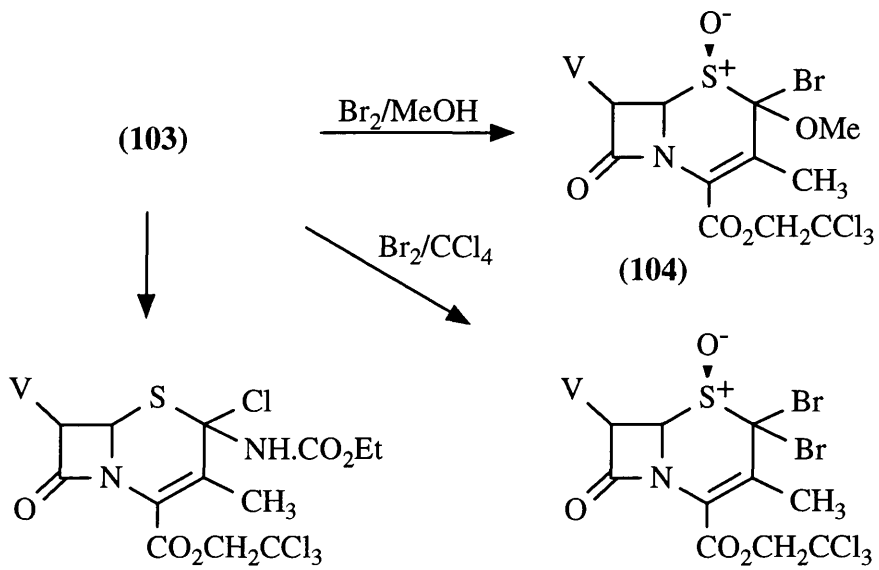


The 2-diazoceph-3-em (**103**) was prepared[59] in a diazo-exchange reaction by brief treatment of sulphoxide (**51**) with triethylamine and tosyl azide . The authors later describe[60] the stability of (**103**) in perchloric acid and glacial acetic acid; nevertheless (**103**) undergoes a range of successful reactions with the halogens and pseudohalogens. Compound (**103**) was transformed to (**104**) in a



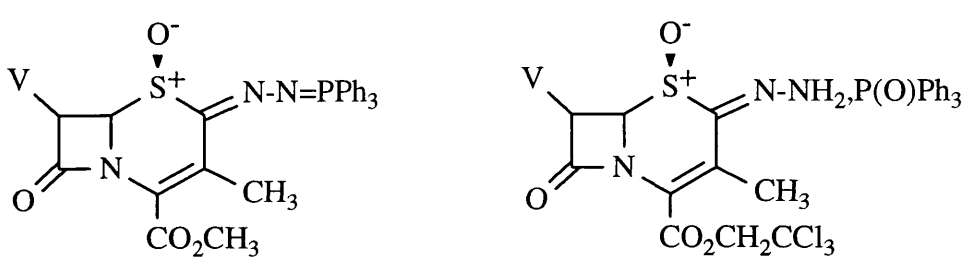
(103)

23% yield with bromine in dry methanol. The 2,2-dibromo adduct **(105)** was obtained in 68% from treatment of **(103)** with bromine in CCl_4 and **(106)** was formed in a yield of 25% from **(103)** and ethyl N-chlorocarbamate.



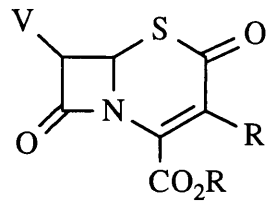
(106) **(105)**

Furthermore, the trichloroethyl ester **(103)** afforded the phosphazenes **(107)** using triphenylphosphine in dry DMF whereas the methyl ester reacted with triphenylphosphine in THF at room temperature to give the phosphine oxide adduct **(108)** in 73%.



(107) **(108)**

Interestingly Rosati *et al*[61] described the conversion of the 2-diazocephem system **(103)** to the 2-oxocephem system **(109a)** by treatment with rhodium acetate. De-esterification by the usual procedure resulted in **(109b)** which exhibited a low order of activity against a wide range of organisms.



(109) a; $R = CH_2CCl_3$

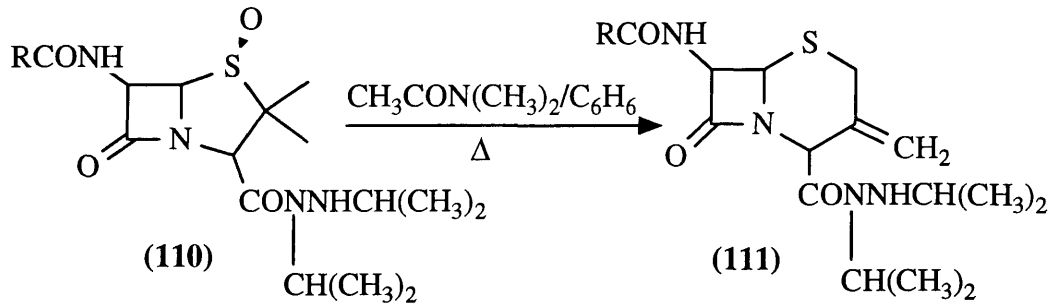
b; $R = H$

1.4 Reactions at C-3

The early 1970's marked the beginning of research on the preparation and biological activity of cephalosporins with electronegative substituents attached to the C-3 position of the dihydrothiazine ring. This was made practicable by the progress of both chemical and electrochemical preparation of 3-methylenecephams by various laboratories. Although 3-exomethylenecephams show no biological activity, they are valuable intermediates to cephalosporins with direct heteroatom substitution at C-3.

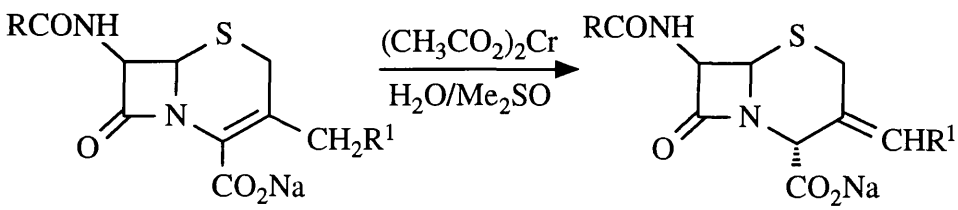
1.4.1 3-Exomethylene

The first productive preparation[62] of 3-exomethylenecepham hydrazides **(111)** was from the rearrangement of the penicillin sulphoxide **(110)** by refluxing with dimethylacetamide-benzene in the presence of an sulphonic acid catalyst.



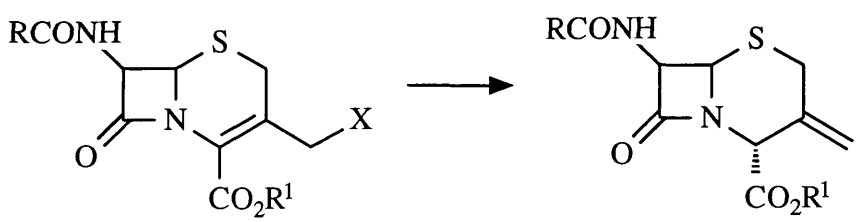
(110) (111)

However the first practical conversion[63] involved treatment of the sodium salt **(112)** with chromium (II) acetate in a 1:1 mixture of H_2O-Me_2SO at room temperature affording the 3-methylene derivatives **(113)**. Surprisingly using the methyl ester or reacting **(112)** in a non-aqueous medium resulted in the starting material being recovered unchanged.



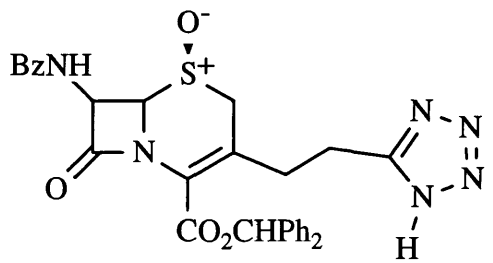
(112) **(113)**

A more recent method[64], which can be easily converted into industrial scale preparation, entails electrolysis of the 3-chloromethylcephems **(114; X=Cl)** in aqueous THF/$LiClO_4$/NH_4ClO_4 and/or MeCN/EtOH/$LiClO_4$/NH_4ClO_4 with a lead cathode and a platinum anode, resulting in reduction to the desired 3-exomethylene cepham **(115)** in high yields. The reduction has also been applied to 3-iodomethyl and 3-benzothiazolyl cephems although in slightly lower yields.



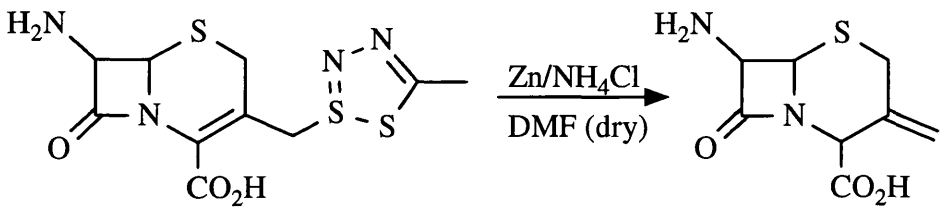
(114) **(115)**

Also, chemical reduction with Zn/AcOH conditions converts the sulphoxide **(116)** into its corresponding exomethylene cepham derivative[65]. In a similiar way 3-exomethylene adducts **(115; R^1=H)** have been produced in 50-80% yields using Zn and NH_4Cl[66].



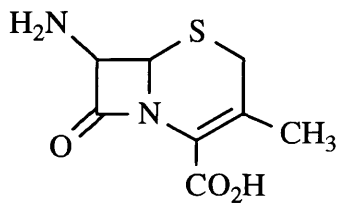
(116)

Kobayashi *et al*[67] (*via* activated Zn/NH_4Cl) carried out an in depth study of the synthesis of 3-exomethylenecephams from **(117)** which contained no C-7 amide or C-4 carboxyl protecting groups. The desired product **(118)** was obtained in high yields (74-85%) along with a minor product **(119)** furnished in 5-10% yield.



(117)

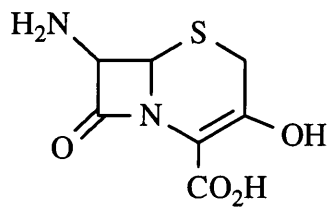
(118) +



(119)

Previous Zn reduction in aqueous acidic conditions (Zn/HCl, Zn/H_2SO_4 or Zn/*p*-TsOH in H_2O or 50% DMSO, MeOH or AcOH) resulted in a higher yield (12-42%) of the isomer **(119)** and 78-44% of **(118)**. Furthermore ozonolysis at -75°C of **(118)**, followed by $NaBH_4$ reduction in aqueous basic conditions resulted

in the corresponding 3-hydroxycephem **(120)**. An increase in reaction temperature to -40°C culminated in oxidation of the sulphur to the sulphoxide **(121)** and hence prevented ozonolysis at C-3 due to steric and electronic hindrance by the sulphoxide moeity.



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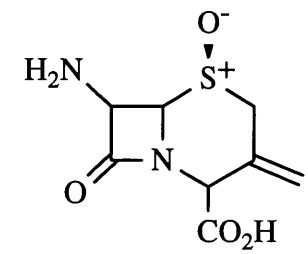
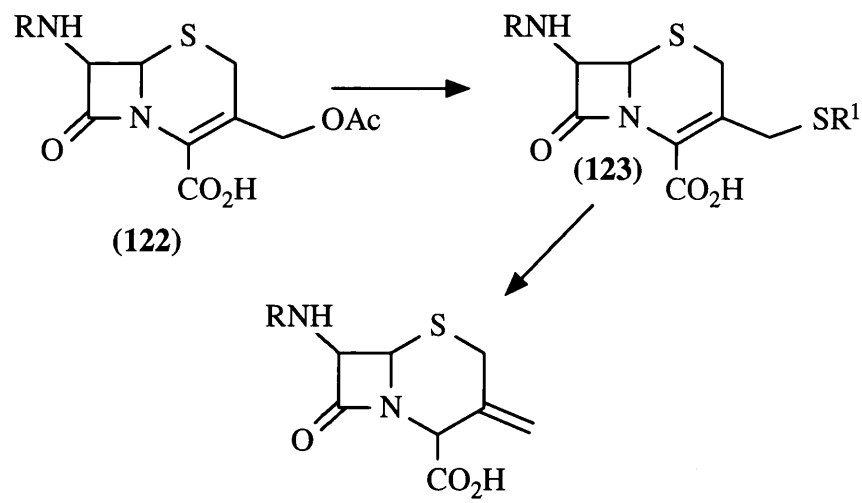


(121)

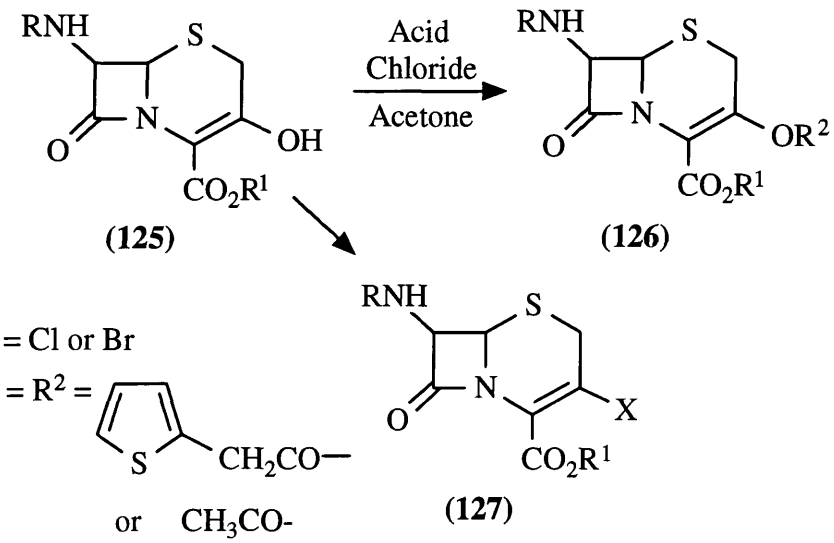
Other chemical reductions have been carried out[68] by displacement of the acetoxy group of cephalosporanic acids **(122)** with sulphur nucleophiles **(123; R^1= COPh, $C(NH_2)_2^+$)** to give the desired 3-exo-methylene compound **(124)**.



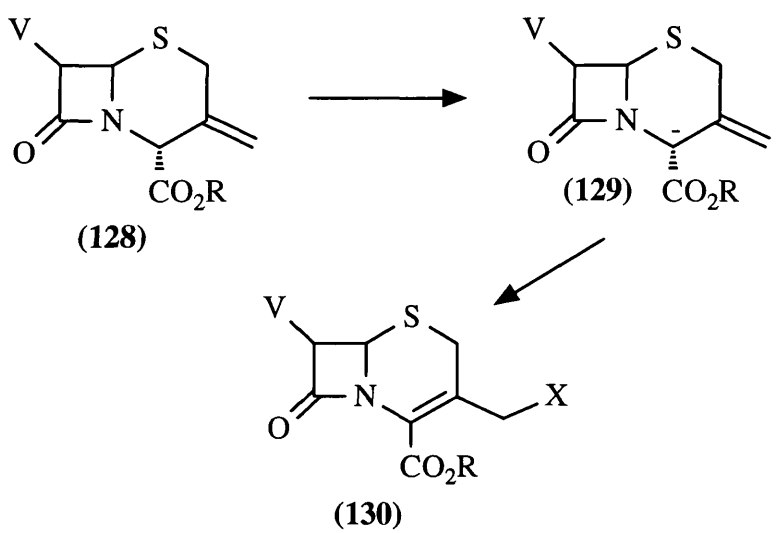
(122) **(123)** **(124)**

The focal point of a later publication[69] by the same authors is the oxidation by low temperature ozonolysis of these products to the 3-hydroxycephems **(125)** and incorporation of a heteroatom at C-3 affording products such as **(126)** and **(127)**. Chlorination was acheived from **(125)** using numerous chlorinating agents ($SOCl_2$, PCl_3, $POCl_3$, $(COCl)_2$ and $COCl_2$) in dry DMF at room temperature. The corresponding 3-bromocephem was obtained from

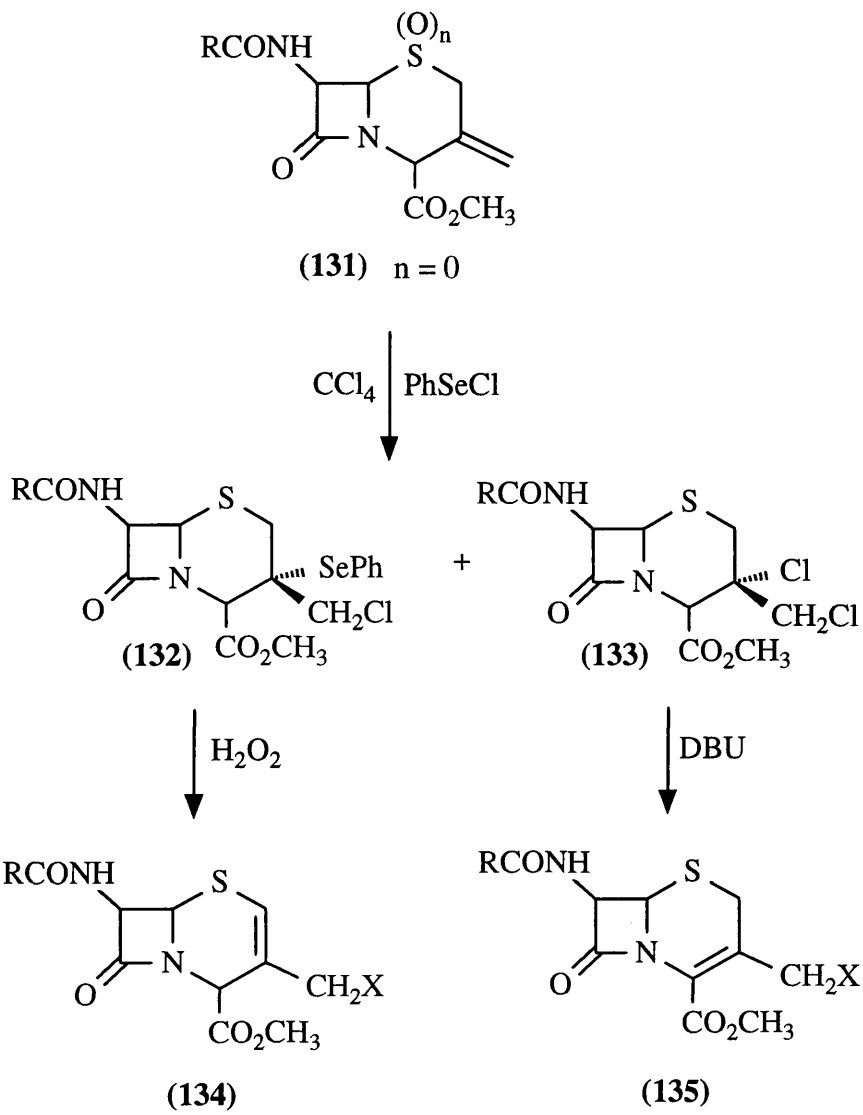
similiar conditions using PBr_3. Also the 3-methoxy derivatives were produced from diazomethane treatment of **(125)** in ether at room temperature. Both the 3-methoxy and 3-halo products exhibited gram-positive and gram-negative activity.



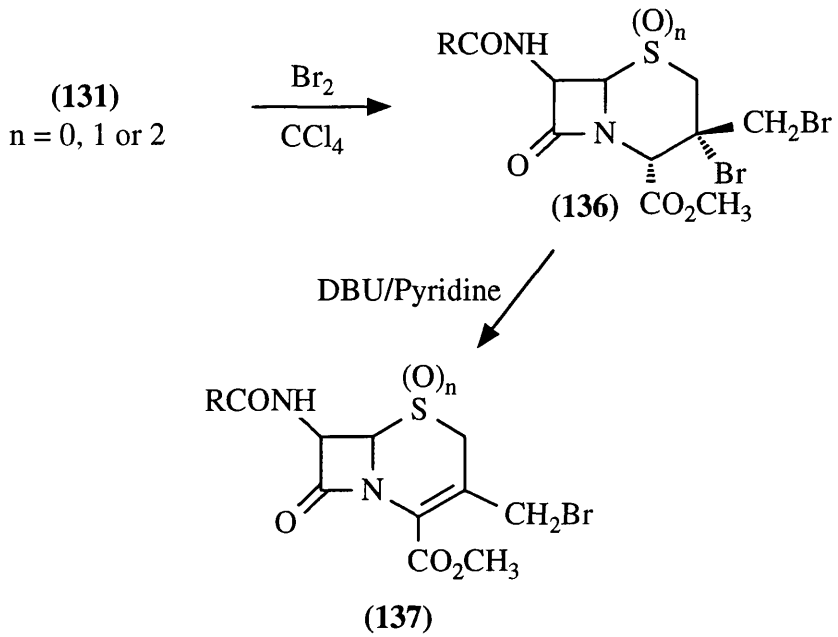
3-Chloromethylcephems **(130; X=Cl)** are also obtained (in 40% yield) from the reaction of 3-exomethylene cepham **(128)**[70]. Lithium methoxide in THF forms an allylic anion **(129)** which is trapped with 2 equivalents of *tert*-butylhypochlorite. The 3-bromomethyl derivatives **(130; X=Br)** were similiarly obtained in 80% yield using 1,5-diazobicyclo-[5.4.0]undec-5-ene (DBU) and bromine.



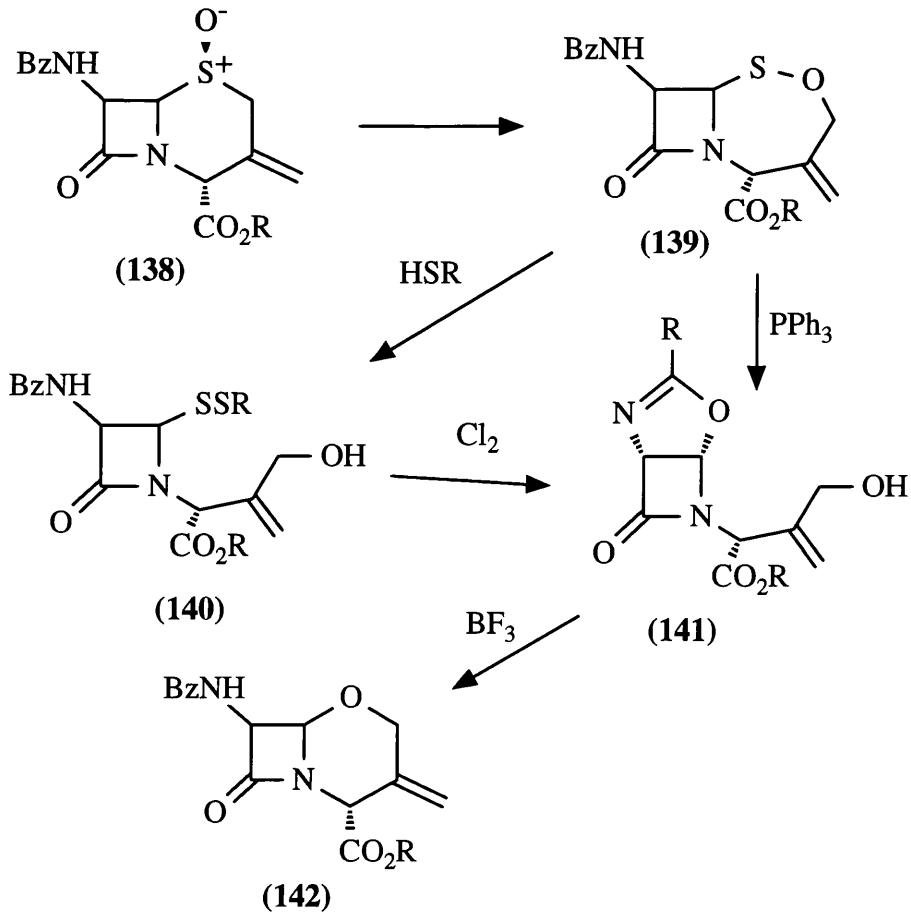
Preparation of 3-chloro and 3-bromo derivatives from 3-methylene cephams have also been studied by Botta and colleagues[71]. Reacting **(131)** in the dark with an excess of phenyl selenyl chloride in CCl_4 afforded 3α-selenophenyl-3β-chloromethyl cepham **(132)** in a yield of 87% and the dichloro adduct **(133)** as a minor product. Hydrogen peroxide oxidation of **(132)** results in elimination of PhSe substituent, creating the 3-chloromethylceph-2-em **(134)**. However in the presence of DBU, **(133)** affords the desired 3-chloromethylceph-3-em **(135)**.



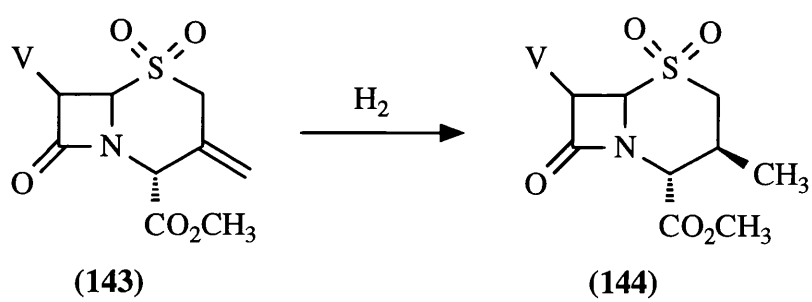
Addition of bromine, not only to the 3-exomethylene sulphide but to its corresponding α-sulphoxide and sulphone, with an excess of bromine at 0°C in chloroform gives the 3β-bromomethyl-3α-bromocephams **(136)** as major products. Elimination of HBr in all cases with DBU/pyridine affords the 3-bromomethyl adducts **(137)**.



Yanagisawa and Ando[72] used the exomethylene cepham **(138)** to prepare the corresponding 3-exomethylene-1-oxacephem **(142)** *via* the disulphide **(140)**, however Cooper *et al*[73] extended this work by eliminating formation of the disulphide and accomplishing a direct conversion of the sulphenate intermediate **(139)** to the oxazolone-azetidinone allylic alcohol structure **(141)** using triphenylphosphine. Transformation of **(141)** was carried out with a 50% solution of boron trifluoride in ether at room temperature affording **(142)** in an overall yield of 27% (higher than the previous report).

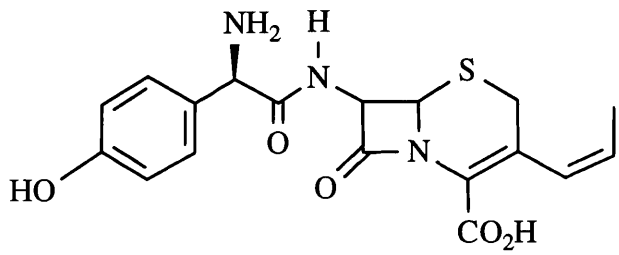


Hydrogenation of 3-methylene cephams has been published by Lilly research laboratories[74], for example using palladium on carbon at low pressure converts the 3-methylene sulphone **(143)** into the single isomer **(144)**.



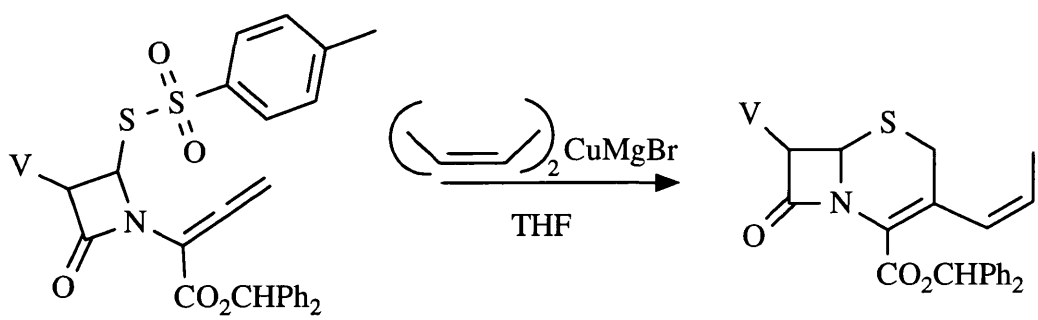
1.4.2 3-Vinyl- and 3-Allylcephalosporins

Discovery[75] of the orally active cephalosporin Cefprozil **(145)** containing the 3-vinyl substituent has provided an impetus for further research[76] in this area.



(145)

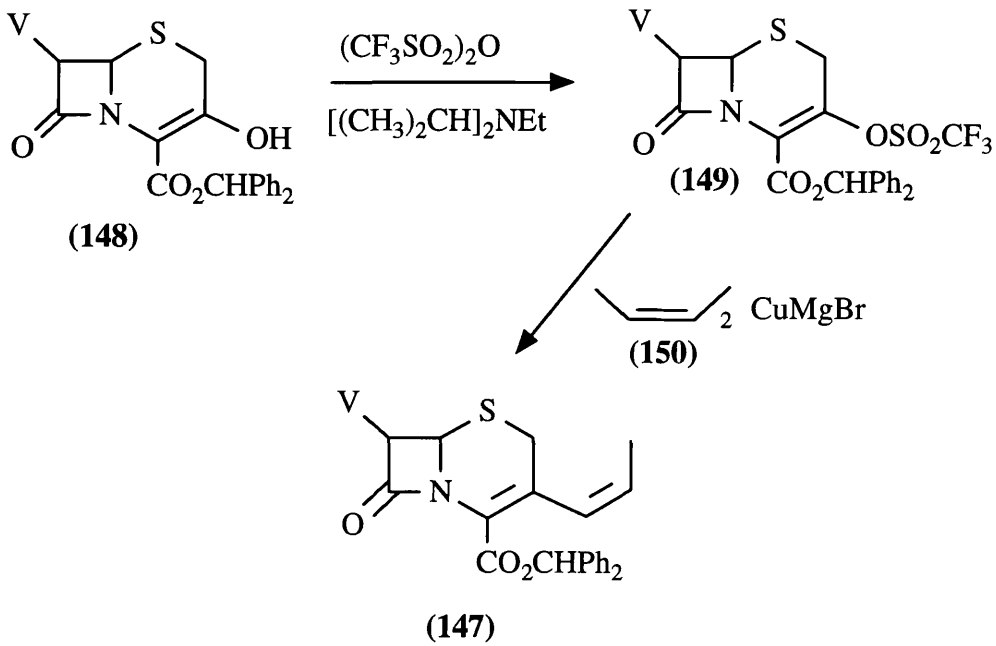
Kant and Farino[77] investigated methods of introducing a vinyl group at C-3 cost-effectively and concisely and described the addition of an organocuprate to an allenylazetidinone to create a carbon-carbon bond followed by ring closure to give the desired 3-vinylcephems **(147)**. Hence treatment of **(146)** with (Z)-1-(propenyl)$_2$CuMgBr (generated from propenyl magnesium bromide and copper iodide) in THF at -100°C afforded the Cefprozil derivative **(147)** in over 96% yield.



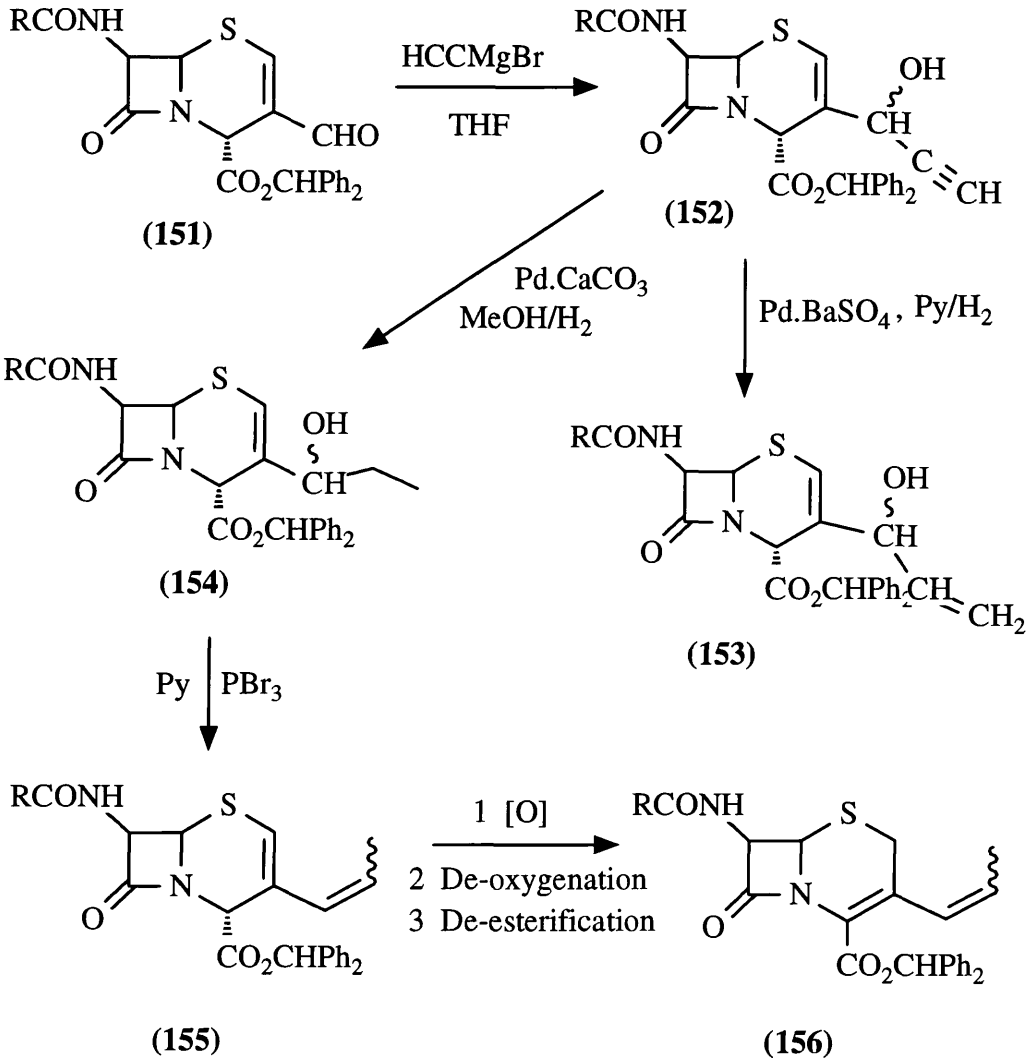
(146) **(147)**

A more recent publication[78] has detailed other efficient methodology to synthesise 3-substituted cephems using organocuprates. 3-Hydroxycephem **(148)** when treated with trifluoromethanesulphonic anhydride and diisopropylethylamine

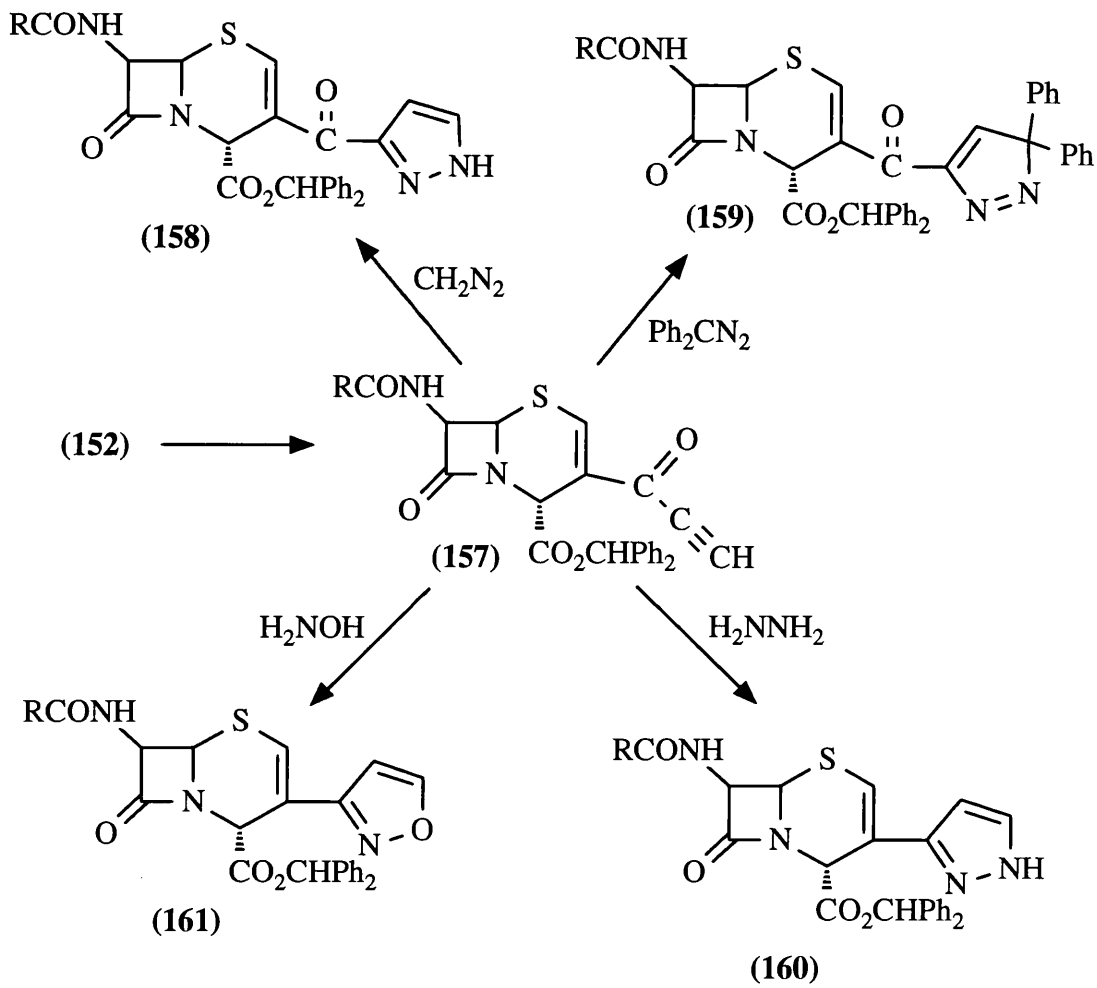
at -78°C gave 90% of a highly crystalline 3-trifloxycephem **(149)** which readily undergoes addition followed by elimination with a wide range of organocuprates (formed from copper (I) bromide-dimethylsulphide complex and an Grignard reagent). Cephem **(149)** therefore, reacted to give **(147)** in the case of cuprate **(150)** in boron trifluoride etherate and THF. As in the previous paper this methodology was applied to a wide range of organocuprates resulting in the desired products in yields over 60%.



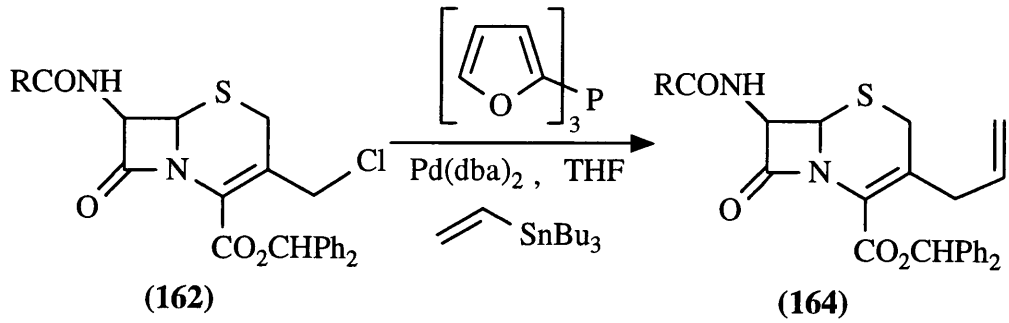
Using 4 equivalents of the alkyne Grignard reagent, ethynylmagnesium bromide, the Δ2-aldehyde **(151)** was converted into the unsaturated alcohol **(152)** which proved to be a fruitful intermediate[79] to other 3-substituted cephems. Reduction with Pd-$BaSO_4$/Py/H_2 afforded the allylic alcohol **(153)** whereas Pd-$CaCO_3$/MeOH/H_2 gave the saturated alcohol **(154)**. Product **(154)** in the presence of PBr_3-pyridine resulted in **(155)** which was de-esterified to give the Δ3-propenyl derivative **(156)**.



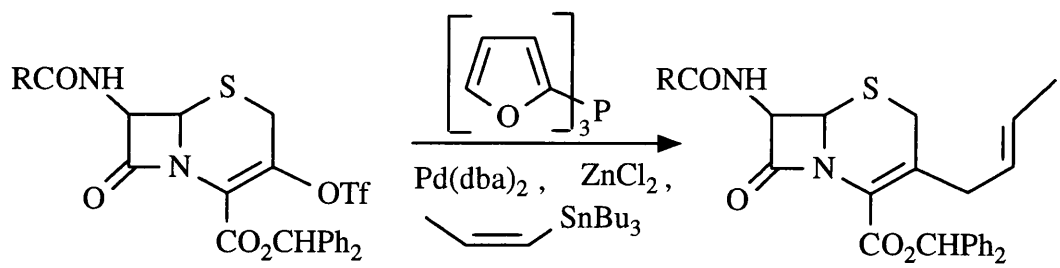
Alternatively **(152)** can be oxidised in good yield with chromic acid to the Δ2-ketone **(157)**, followed by a 1,3-dipolar cycloaddition using diazomethane (50%) or diphenyldiazomethane (87%) to the pyrazoles **(158)** and **(159)** respectively. Ketone **(157)** also reacts with hydrazine affording **(160)** and hydroxylamine to give the isoxazole **(161)**. All corresponding Δ3-acids exhibited poor biological activity.



The preparation of 3-allyl substituted cephems was also recently described[80]. A Pd catalysed coupling reaction between either 3-chloromethylcephems **(162)** or 3-triflyloxycephems **(163)** and unsaturated stannanes resulted in a variety of derivatives with the general structures **(164)** in 57-82% yields and **(165)** in 50-79% yields. The most effective catalytic system for cephems **(162)** was prepared by adding tri(2-furyl)phosphine to a THF solution of bis(dibenzylideneacetonyl)palladium and similiarly for **(163)** but using N-methylpyrrolidine (instead of THF) in the presence of $ZnCl_2$.



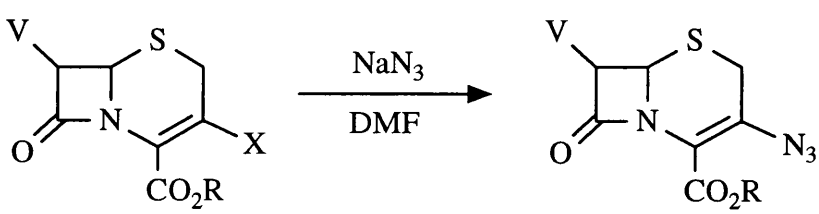
(162) (164)



(163) (165)

1.4.3 3-Azidocephems

The diverse reactions of azides in organic chemistry eg with electrophiles, nucleophiles and dipolarophiles and their easy preparation in cephalosporin chemistry from displacement of either chloro or tosylate substituents has led to preparation and investigation into C-3 azidocephems. Cephem **(166)** was converted into the 3-azidoceph-3-em **(167)** in the presence of Na_3N in DMF and delocalisation of the azide electrons was maximised by Δ3-configuration[81].

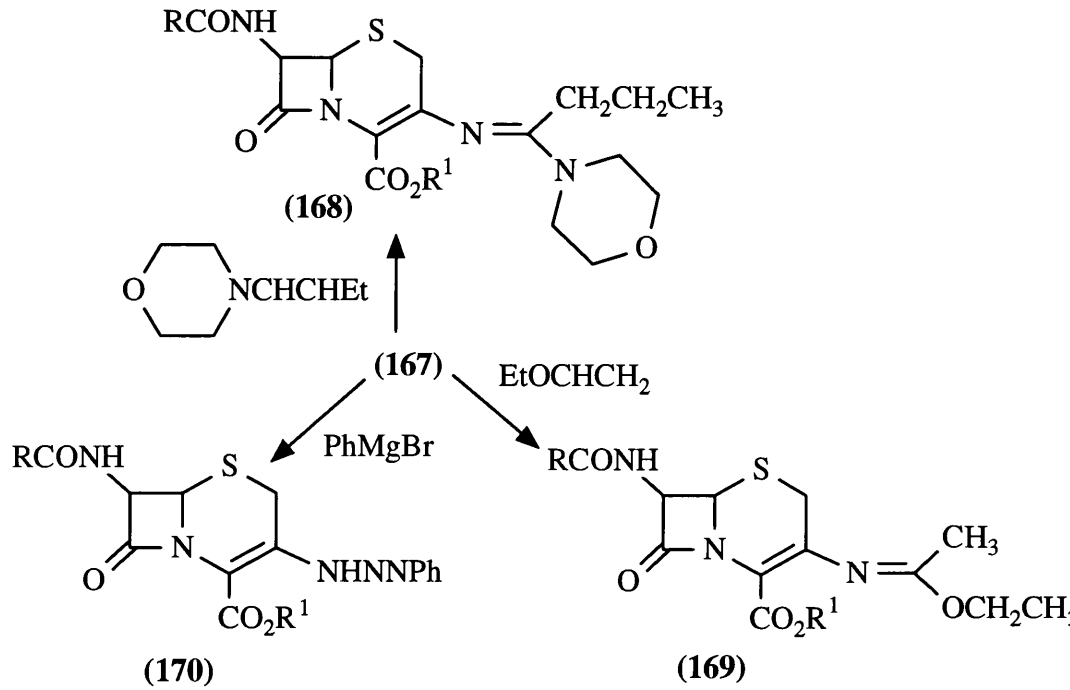


(166) **(167)** 80-90%

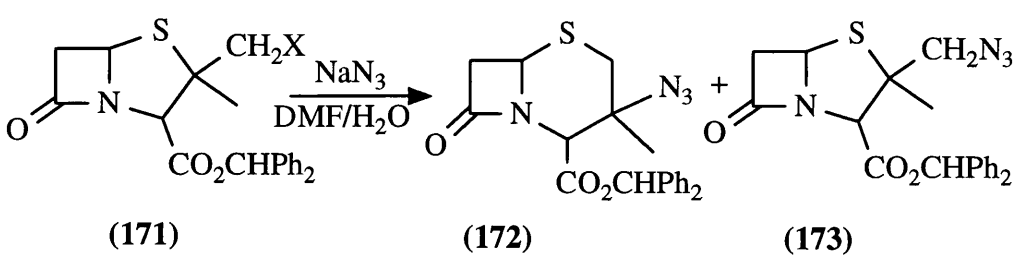
R = CH_3, $CHPh_2$ and CH_2CCl_3

As a result, an electron deficient azide is created and reactivity is lowered. Hence further reaction to amidines **(168)** and imidates **(169)** can only be completed with

electron rich olefin species. Triazines (**170**) are also synthesised from reaction of (**167**) with Grignard reagents. Reduction of (**167**) afforded the acid (**167**; R^1=H) which exhibited poor biological activity.



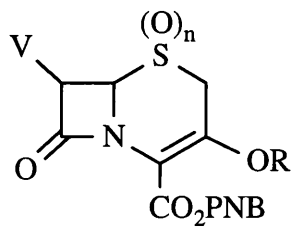
Additionally 3-azidocephams (**172**) along with 2-azidomethylpenams (**173**) have been prepared in a 2:3 ratio from 2-halomethylpenams (**171**) in the presence of sodium azide (NaN_3) in aqueous DMF[82]. Aqueous acetone and acetonitrile have been used in place of DMF however they lengthened reaction time. Temperature too has also been varied and although this had no effect on product ratio, it affected the rate of reaction. In some cases only starting material or decomposition products were isolated.



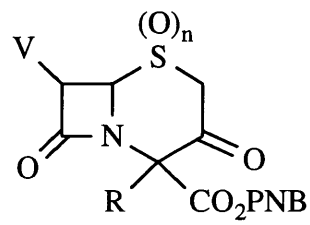
(**171**) (**172**) (**173**)

1.4.4 3-Alkoxy- and 3-Alkylcephems

Spry *et al*[83] have described a novel synthesis of 3-alkoxycephems from their analogous 3-hydroxy derivatives *via* a Mitsunobu reaction. Thus, reacting the enol (**174; R=H**) in THF or CH_2Cl_2 at room temperature for 20 mins with a series of alcohols in the presence of triphenylphosphine and dimethyl azodicarboxylate resulted in the desired O-alkylated product (**174**) in 60-90% yields and 10-15% of the minor 4-alkylated product (**175**).



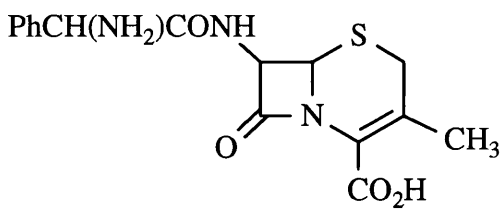
(**174**)



(**175**)

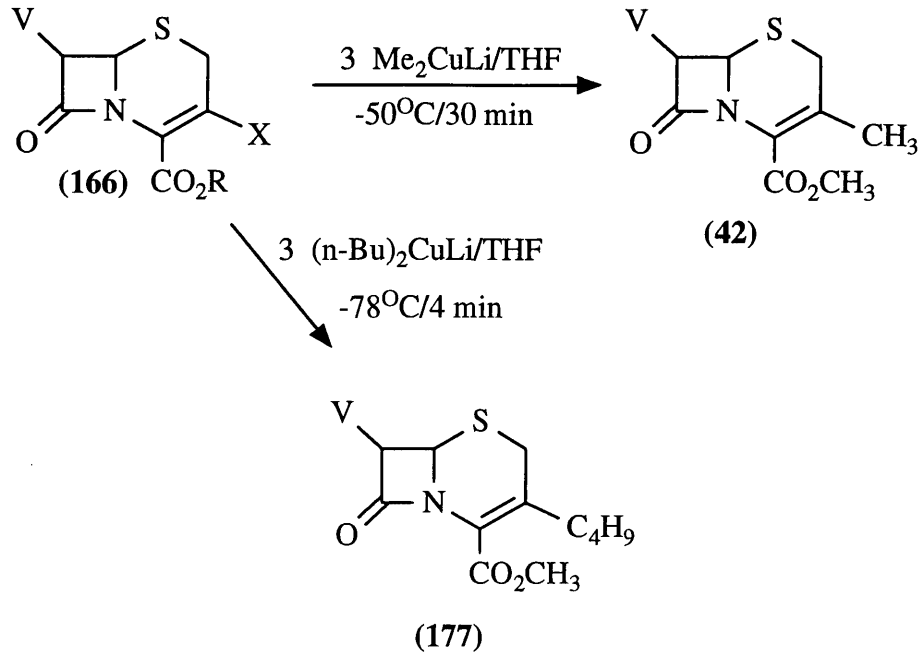
R = Me, Et, $CHPh_2$ and n-Bu

Biological studies have shown that a number of these 3-alkoxy derivatives possess activity, some even displaying similar activity to cephalexin (**176**).

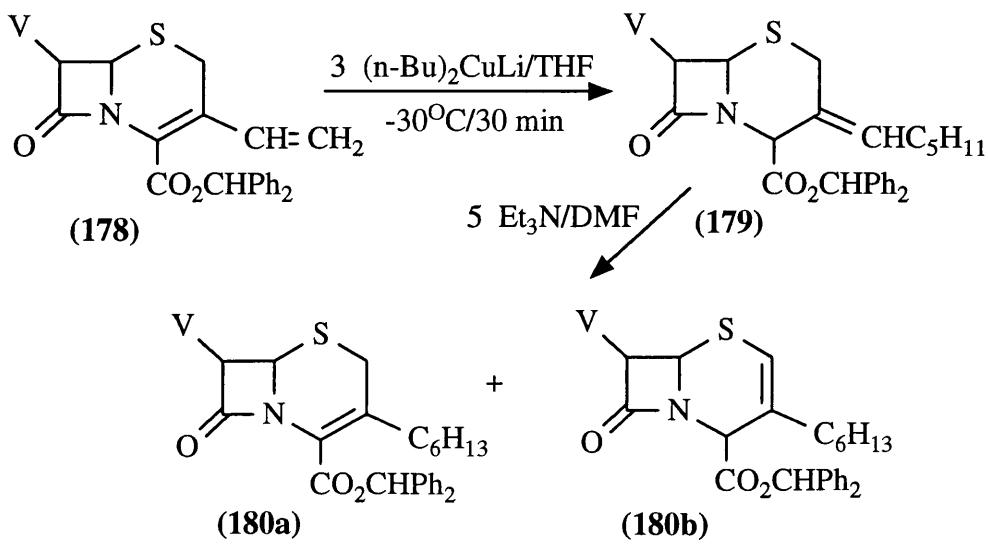


(**176**)

Alkylation of 3-chloro and 3-vinyl cephems using the appropriate organocuprate was also reported by Spry and Bhala[84]. 3-Chlorocephem (**166; R=CH_3 and X=Cl**) reacted in the presence of either lithium dimethylcuprate or lithium di-*n*-butylcuprate to give (**42**) and (**177**) respectively. The 3-vinylcephem



(178) afforded exocyclic alkene **(179; 82%)** on reaction with lithium di-n-butylcuprate followed by conversion to the 3-alkylated derivatives **(180a)** and **(180b)**. Spry deduced[84] that 'increasing the lipophilic character at C-3' seems to



enhance activity towards gram-positive bacteria while diminishing the activity towards gram-negative bacteria.

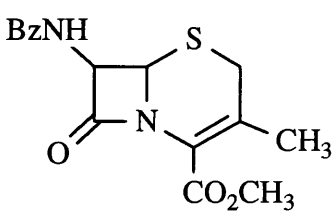
1.5 Reactions at C-4

Incorporating or varying substituents at the 4-position in cephalosporin chemistry has been achieved *via* three main pathways, ie addition of groups to C-4 by a base generated carbanion creating 4-disubstituted ceph-2-ems; addition across the Δ-3 double bond affording 3,4-disubstituted cephams and direct modification of the C-4 carboxylic acid.

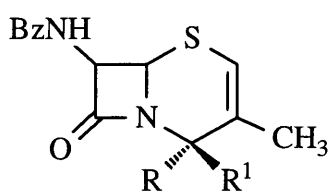
1.5.1 Δ2-Cephems

Numerous publications have reported the introduction of groups at C-4 resulting in a Δ2-double bond and an α-orientated carboxylic acid.

Using base conditions at low temperatures, Yoshida, Oida and Ohki[85] investigated substitution reactions of the 7-acylaminoceph-3-em derivative **(181)**. Reaction with methyl methanethiosulphonate in the presence of lithium diisopropylamide in THF afforded just one of two possible isomers at C-4, specifically the 4β-methylthiocephem **(182a)** in 53% yield. Using methylsulphenyl chloride as the methylthiolating agent the reaction proceeds less stereospecifically forming 30% of **(182a)** and 13% of the α-isomer: 4α-methylthiocephem **(182b)**.



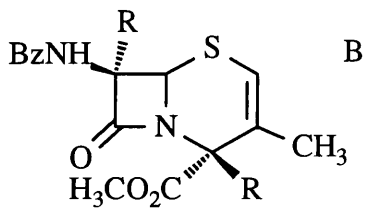
(181)



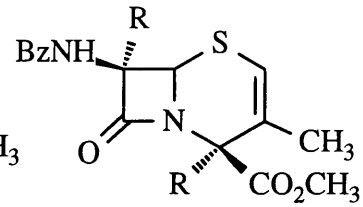
(182) a; $R = CO_2CH_3$, $R^1 = SCH_3$
b; $R = SCH_3$, $R^1 = CO_2CH_3$
c; $R = CO_2CH_3$, $R^1 = CH_3$
d; $R = CO_2CH_3$, $R^1 = PhCH_2$

A later publication[37] reports the alkylation of **(181)** with 2 equivalents of lithium diisopropylamide in THF with methyl iodide resulting in β-oriented 4-methylcephem **(182c)** in 55% yield. An analogous reaction of **(181)** with an

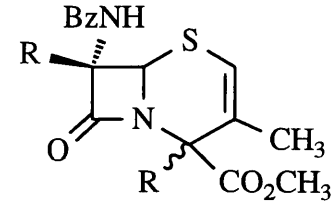
extra equivalent of base gave a complex mixture of dimethylated products **(183a)**, **(184a)** and **(185a)** as well as **(182c)** in 14, 8, 19 and 17% yields respectively.



(183) a; R = CH_3
b; R = $PhCH_2$



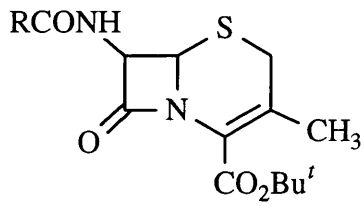
(184) a; R = CH_3
b; R = $PhCH_2$



(185) a; R = CH_3
b; R = $PhCH_2$

Alkylation of **(181)** with benzyl bromide was accomplished in a similar manner to methylation. Two equivalents of base resulted in the 4β-benzylcephem **(182d)** in a 47% yield. In the presence of three equivalents of LDA the formation of 4β,7α-dibenzylceph-2-em **(183b)** predominated (43%). Cephems **(184b)** and **(185b)** were formed as by-products in 10% and 13% yields.

Methylthiolation[86] of the cephem nucleus **(186)** was carried out using lithium N-cyclohexylisopropylamide and $MsSCH_3$ to afford 4β-substituted products **(187)** in 30-60% yields. Deprotection gave the 4-methylthiolated free acids **(187; R^1=H)** which exhibited no significant antibacterial activity.



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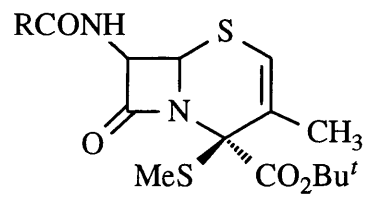
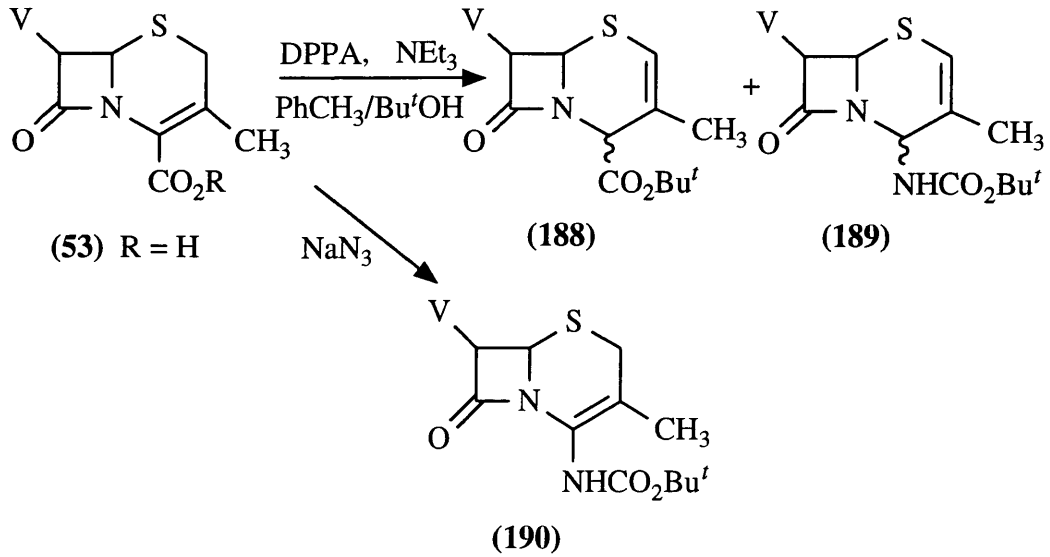


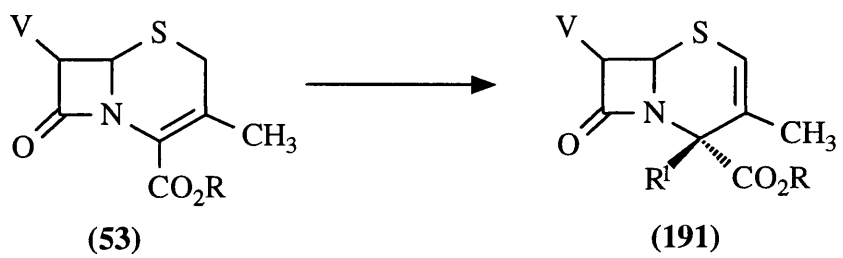
(187)

Curtius reaction conditions have been reported[87] as a method for modifying the 4-position of cephalosporin **(53)**. Reaction of cephem **(53)** with diphenylphosphorylazide (DPPA) and triethylamine in a heated solution of toluene/*t*-butyl alcohol furnished the 4α-ester **(188)**, the 4β-ester **(188)** and the 4*t*-butoxyformamidocephem **(189)** in 18%, 3% and 11% yields respectively. In the

presence of excess sodium azide (**53**) was converted into a 14% yield of (**190**) as the only β-lactam product regardless of whether or not NEt_3 was used.



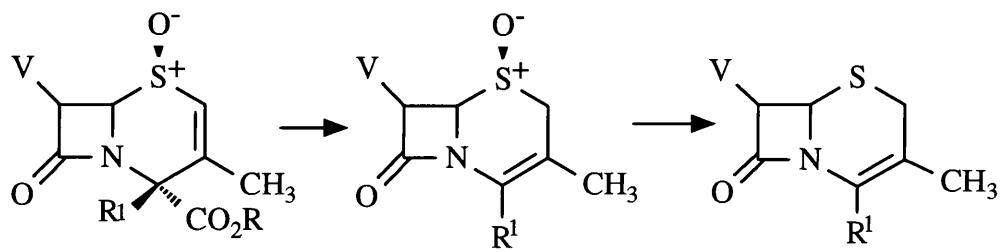
Finally Michael-type additions have also been used[88] in the preparation of 4-substituted cephalosporins using a variety of Michael acceptors. Ceph-3-ems (**53**) were treated with a catalytic amount of triethylamine in methyl vinyl ketone to give the Michael adducts (**191**; **R^1=CH_2CH_2COMe**) in 51-90% yields.



(**53**) (**191**)

Similiarly the diphenylmethyl ester (**53**; **R=$CHPh_2$**) reacted with both acrylonitrile and dimethyl butynediote affording the corresponding Michael adducts (**191**; **R^1=CH_2CH_2CN**) in 74% and (**191**; **R^1=$CH(CO_2Me)CH_2CO_2Me$**) in 40% yields. No reaction was observed between (**53**) and methyl acrylate in the presence of a triethylamine base, however using Triton-B a complex mixture was obtained. Isolation of (**191**; **R=$CH_2CH_2CO_2Me$**) was acheived after oxidation and the corresponding sulphoxide was obtained in 53% yield. De-oxygenation afforded the expected sulphide in 80% yield.

To enable decarboxylation, the ceph-2-em sulphides (**191**) were converted into their corresponding sulphoxides (**192**) using *m*-CPBA. Thus, de-esterification using trifluoroacetic acid/anisole in DMF for diphenylmethyl esters and Zn/acetic acid in DMF for trichloroethyl esters was followed by spontaneous decarboxylation and resulted in novel 4-monosubstituted cephems (**193**) which were de-oxygenated to give (**194**).

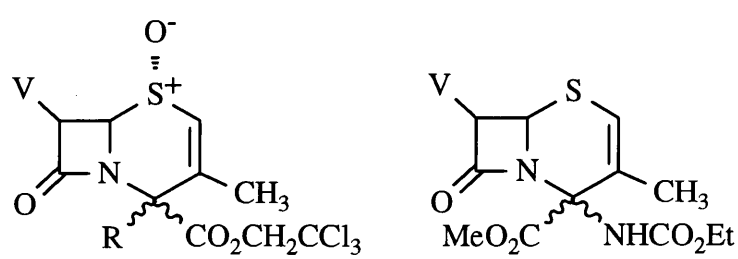


(**192**) (**193**) (**194**)

R^1 = -CH_2CH_2CN, -$CH(CO_2Me)CH_2CO_2Me$
and $CH_2CH_2COCH_3$

During investigations into Michael additions it was also reported[59] that R-sulphoxides result in the addition taking place at the C-4 position affording products such as (**195**). This is explained by steric factors ie the 7β-side chain and α-sulphoxide moiety hinder the addition at C-2β and C-2α respectively.

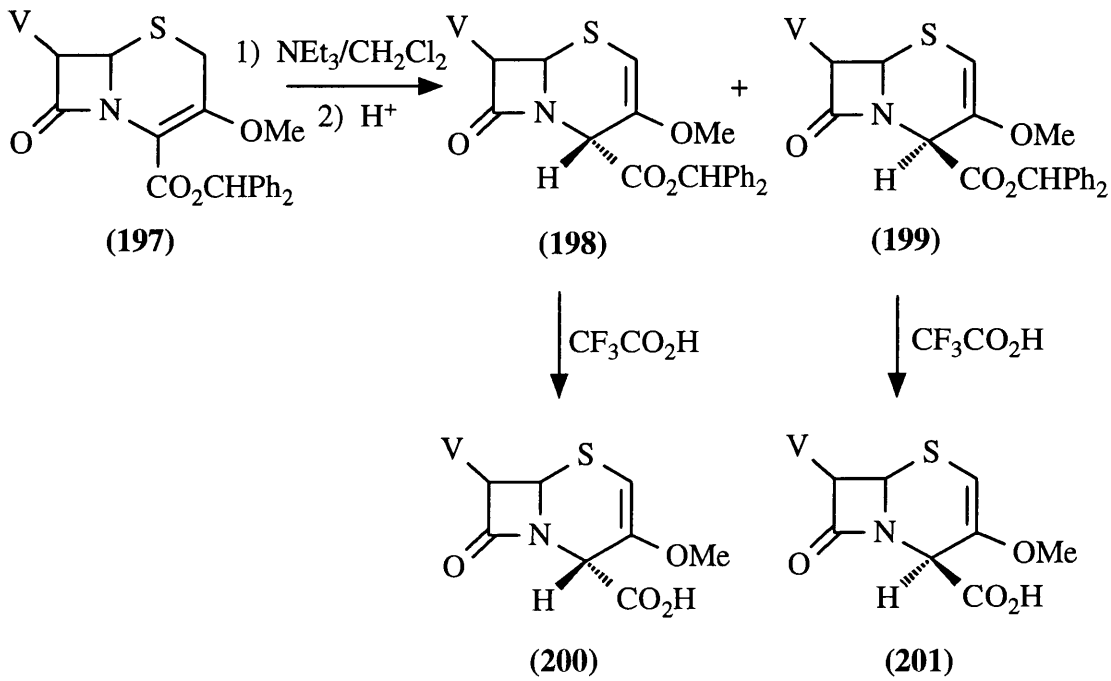
The same authors also report[89] reaction of the methyl ester (**53; R=Me**) with N-chloro-N-sodiourethane in methanol/acetonitrile to give (**196**) a novel 4-substituted cephem isolated in 17% yield.



(**195**) R = CH_2CH_2CN (**196**)

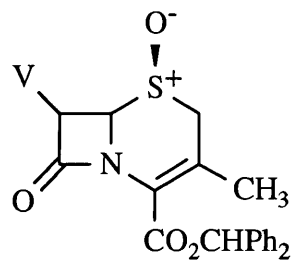
Isomerisation to a ceph-2-em in which the carboxylic acid becomes α-orientated results in diminished antibacterial activity. Frere *et al*[90], who

completed an in depth study into the chemical reactivity of a wide variety of β-lactams, disagreed with initial theories which suggested that the β-lactam moiety of ceph-2-ems had less acylating power than their ceph-3-em counterparts. Cohen[91] inspired by Frere and colleagues, investigated 3-D models of both active and inactive β-lactam structures and concluded that the biological inactivity of ceph-2-ems is the result of a misfit with the 'highly specific 3-D recognition sites of the transamidase enzymes and a direct consequence of the α-orientated carboxylic acid group'. Nevertheless he proposed that 4β-isomers would fulfil the 3-D requirements and hence possess biological activity. In an attempt to ascertain whether his theories were correct, the C-4 carboxylic acid isomers **(198)** and **(199)** were prepared by equilibration of the 3-methoxyceph-3-em ester **(197)** in CH_2Cl_2 using triethylamine. Deprotection and isolation afforded the corresponding acids **(200)** and **(201)**, which displayed no significant biological activity and certainly less so than the corresponding Δ3-cephems.

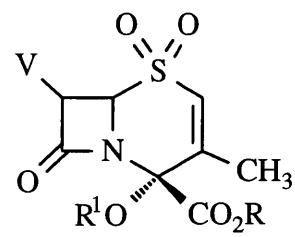


The preparation of additional 4α-carboxylic acids by an oxidative rearrangement has been published by Stoodley and colleagues[92]. The

corresponding sulphone of **(202)** reacted with 5% Pd/C catalyst in ethyl acetate to afford the hydroxy derivative **(203a)** which on treatment with acetic anhydride/pyridine was converted to the acetate **(203b)**. De-esterification by standard methods gave the acid **(203c)** which was transformed into its sodium salt **(203d)**. As in Cohen's β-orientated molecules, ceph-2-em sulphone **(203d)** displayed no biological activity.



(202)



(203) a; $R = CHPh_2$, $R^1 = H$
b; $R = CHPh_2$, $R^1 = Ac$
c; $R = R^1 = H$
d; $R = Na$, $R^1 = H$

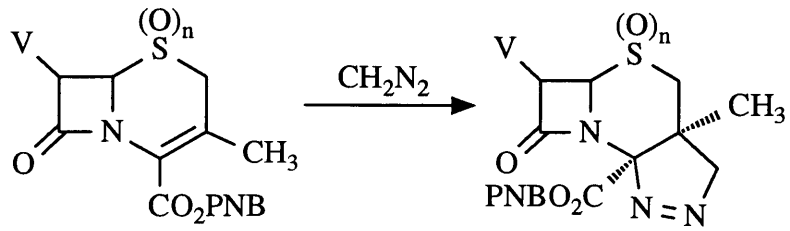
Under similar conditions the sulphoxide **(202)** and sulphide of **(202)** failed to react indicating that the sulphone and ester moieties are important structural features for an oxidative rearrangement to occur.

1.5.2 Addition to the Δ3-Double Bond

In cephems, the Δ3-double bond is incorporated into an enamide system, is α,β- to a carboxyl group and is tetra-substituted. Thus the double bond tends to be unreactive as a result of the surrounding electronic effects and consequently few addition reactions have been reported.

Jaszberenyi and coworkers[93&94] investigated the cycloaddition reactions of the 3-methylcephem **(204)** and its oxides with diazomethane. In all cases the β-orientated pyrazolinocephams **(205)** were obtained because ring strain of the α-face adduct is stronger than that of the β-face. Furthermore no cycloadducts were observed with Δ2-cephems or when the hydrogens of the diazo-compound

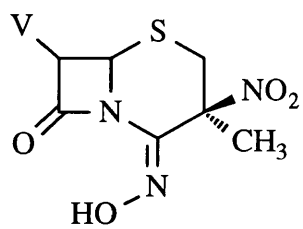
were replaced with carbonyl functions, eg ethyl diazoacetate, which reduce reactivity of the diazo-compound.



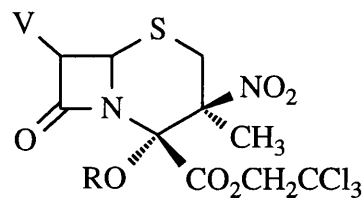
(204) **(205)**

n = 0, 1 or 2

Dinitrogen trioxide is reported[95] to add across the double bond of the corresponding cephalosporanic acid of **(53)** in CH_2Cl_2 at room temperature affording **(206)** in a 67% yield as the Z-isomer. Under similar conditions[96] the ester **(53; R=CH_2CCl_3)** was converted to two unstable β-lactams separated by their solubility in ether. The more stable was obtained in yield of 50% and identified as **(207a)** which was further reacted with acetyl-chloride/triethylamine to afford **(207b)**.

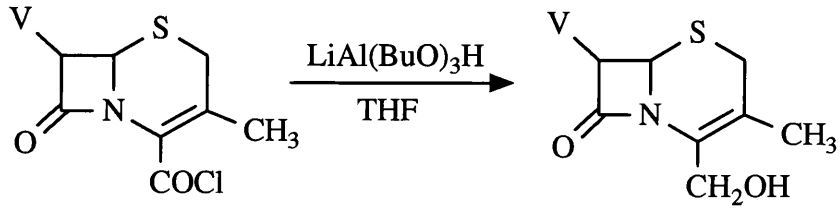


(206)



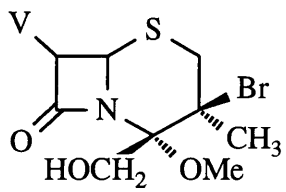
(207) a; R = H
b; R = Ac

As the Δ3-cephem double bond appeared to be unreactive towards many electrophilic reagents[97], Macchia *et al*[98] substituted the C-4 ester function with an hydroxymethyl group to reduce the electron withdrawing effect and consequently increase the electrophilic reactivity of the double bond. Compound **(209)** was synthesised by the reduction of the acid chloride **(208)** with $LiAl(BuO)_3H$ in THF at 0°C. Treatment with bromine in methanol converted

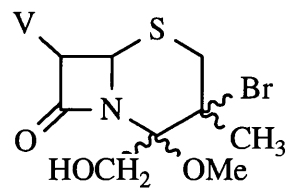


(208) **(209)**

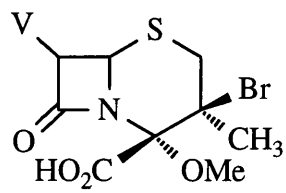
(209) into two bromomethoxy adducts **(210)** and **(211)** in a 6:1 ratio. Oxidation of the 4β-hydroxymethyl analogue **(210)** with a large excess of pyridinium dichromate in DMF with a small quantity of water afforded the cepham derivtive **(212)** which displayed poor antibacterial activity[99]. In addition, the *p*-nitrobenzoyl



(210)

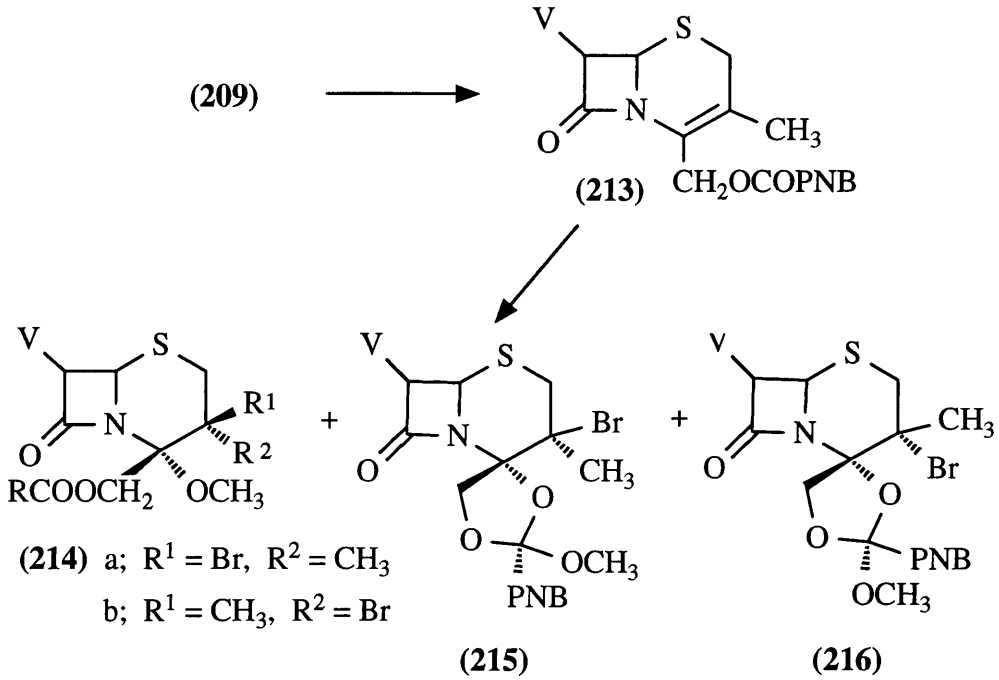


(211)



(212)

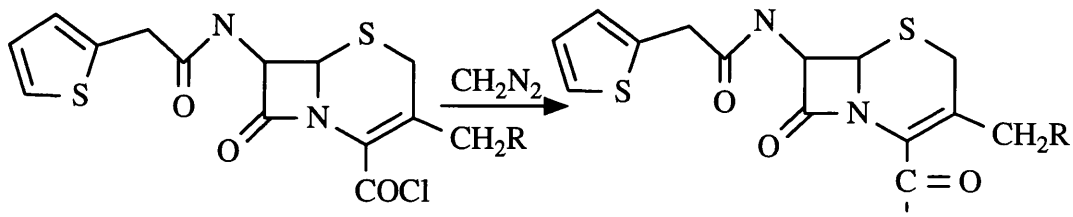
ester **(213)** was synthesised in a 74% yield by treatment of **(209)** with *p*-nitrobenzoyl chloride in pyridine[100]. A similar reaction with bromine in methanol gave the bromomethoxy adducts **(214a)** and **(214b)** in 3% and 2% yields respectively and the spirodioxolane derivatives **(215)** and **(216)** in 8.1% and 5.4% yields.



1.5.3 Reactions at C-4 Carboxyl Group

The carboxyl function at position 4 reacts in a comparable manner to a typical carboxylic acid but other competitively and highly reactive positions of the dihydrothiazine ring system coupled with double bond isomerisation and steric hindrance of the C-3 moiety repeatedly prevent high yields from being obtained from these reactions. Furthermore a free carboxyl group seems to be essential for activity[9].

SmithKline and French laboratories[101] researched the structural requirements at the C-4 carboxyl group and attempted to replace it with other polar moieties. Preparation of the acid chlorides **(217a)** and **(217b)** from the corresponding acids was accomplished with oxalyl chloride in DMF and conversion to the functional intermediate diazo ketones **(218a)** and **(218b)** in 83% and 43% yields respectively with diazomethane in ether. Reduction of **(218b)**

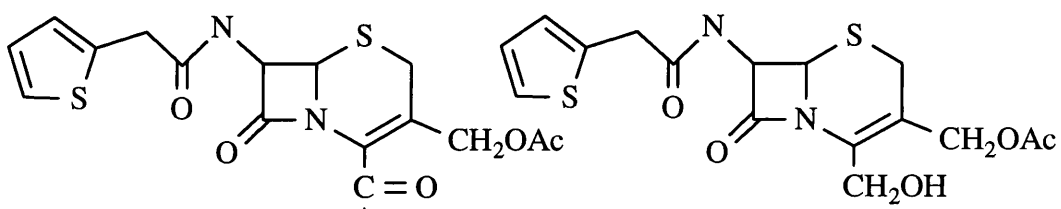


(217) (218)

a; R = H

b; R = OAc

with concentrated HI in chloroform afforded the methyl ketone **(219a)** and application of ethereal HCl, as an alternative, gave the chloromethyl ketone **(219b)** in a 49% yield. The acetoxymethyl derivative **(219c)** was obtained in a yield of 49% by warming **(218b)** in AcOH and **(219d)** was produced in 44% yield *via* methanolysis of **(218b)** with $BF_3.2Et_2O$ in methanol. Additionally reduction of acid chloride **(217b)** to alcohol **(220)** was accomplished with $LiAl(t\text{-}BuO)_3H$. All of these modifications at C-4 carboxyl group resulted in cephalosporins with much less activity than the analogous carboxylic acids.



(219) a; R = CH_3

b; R = CH_2Cl

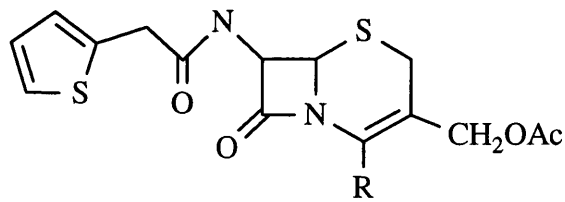
c; R = CH_2OAc

d; R = CH_2OCH_3

(220)

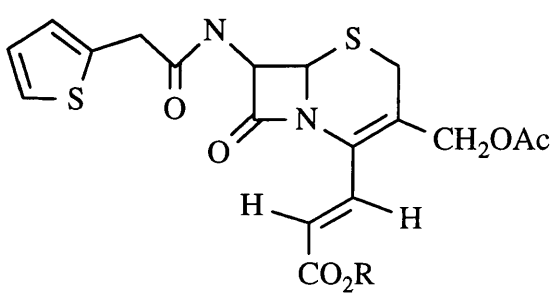
A variety of polar derivatives were prepared from the aldehyde cephem **(221a)**[102]. Commencing from the known alcohol **(220)**[101] the desired aldehyde **(221a)** was obtained *via* a mild Moffatt oxidation (DCC in DMSO and dichloroacetic acid). The oxime **(221b)**, methoxime **(221c)**, semicarbazone **(221d)** and carboxymethoxime **(221e)** were obtained in 34%, 45%, 20% and 30% yields respectively when the aldehyde was dissolved in THF and treated with the

appropriate reagent ie hydroxylamine hydrochloride, methoxylamine hydrochloride, semicarbazide hydrochloride and carboxymethoxylamine hemihydrochloride. Reaction of **(221a)** with diphenylmethoxycarbonylmethyl-

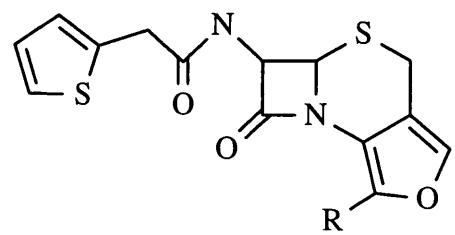


(221) a; R = CHO
b; R = CH=NOH
c; R = $CH=NOCH_3$
d; R = CH=NNHCONH
e; R = $CH=NOCH_2CO_2H$

enetriphenylphosphorane afforded **(222a)** in 40% yield which on removal of the carboxyl protecting group and treatment with sodium 2-ethylhexanoate furnished the sodium salt **(222b)** in 58%. The furan **(223a)** was also attained in 60% yield from the 3-hydroxy aldehyde **(221a)** by stirring at room temperature in 1:1 dioxane:HCl and further converted to **(223b)** in a 40% yield when subjected to the Vilsmeier reaction ($DMF/POCl_3$)[103]. These novel cephalosporins exhibited considerably reduced biological activity compared to cephalothin **(11)**.

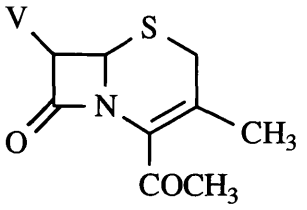


(222) a; $R = CHPh_2$
b; R = Na



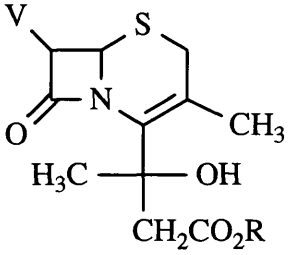
(223) a; R = H
b; R = CHO

Macchia *et al*[104] modified the 4-position by application of the Reformatski reaction of the methyl ketone **(224)**. Using ethyl α-bromoacetate in

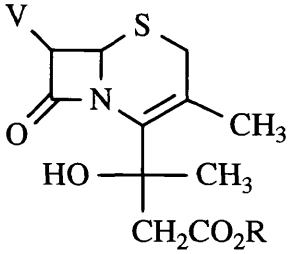


(224)

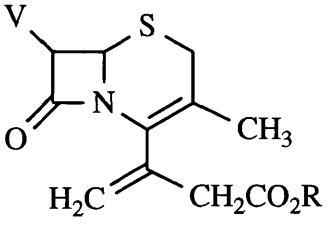
anhydrous benzene, **(224)** was converted to the diastereoisomeric β-hydroxy esters **(225a)** and **(226)**. A similiar reaction using *t*-butyl α-bromoacetate in THF resulted in the hydroxy ester **(225b)** which on treatment with triethylamine gave **(227)**, **(228a)** and **(228b)**. Hydrolysis with 99% formic acid gave the corresponding free acids of **(225b)**, **(227)**, **(228a)** and **(228b)**. Again no biological activity was displayed with these novel cephems.



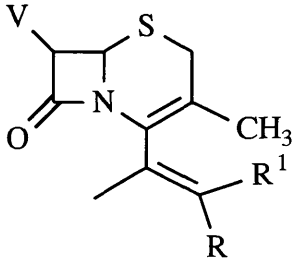
(225) a; R = Et
b; R = Bu^t



(226) R = Et



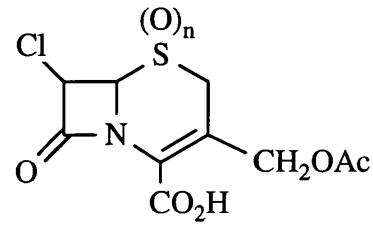
(227) R = Bu^t



(228) a; R = Bu^t, R^1 = H
b; R = H, R^1 = Bu^t

Various spiroazetidinylcephems were prepared from reaction of the sulphide **(229)**, and its various oxides with 2 mol equivalents of DCC in ethanol

free CH_2Cl_2[105]. Sulphide (n=0) required the presence of triethylamine to give a 4% yield of **(230; n=0)** and the sulphone (n=2) gave **(230; n=2)** quantitatively. The α-sulphoxide reacted non-stereoselectively and produced both epimers **(230; n=1)** and **(231)** in comparable amounts whereas the β-sulphoxide **(229; n=1)** was converted smoothly to **(230; n=1)** in 74% yield. The sulphones **(232a and b)** were



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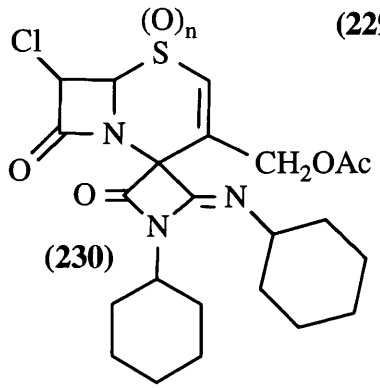
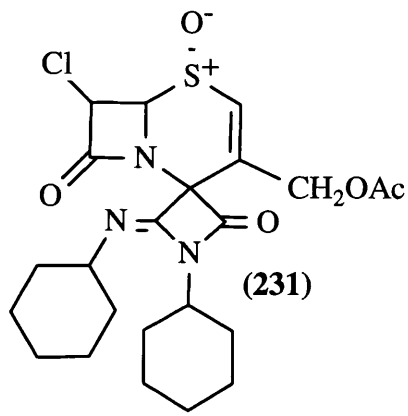
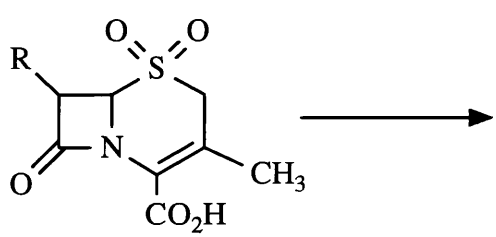


(230)

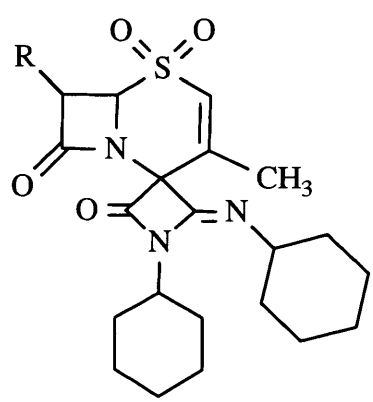


(231)

also treated with DCC to assess the scope of the reaction and the isolated products were identified as the desired 4-spiro-adducts **(233a and b)** obtained in 52% and 20% yields respectively.

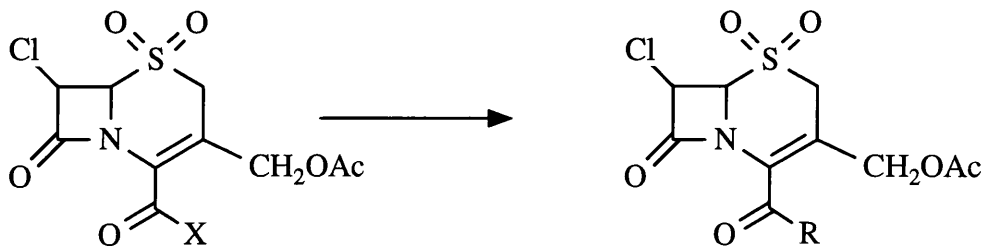


(232) a; R = H
b; R = V



(233) a; R = H
b; R = V

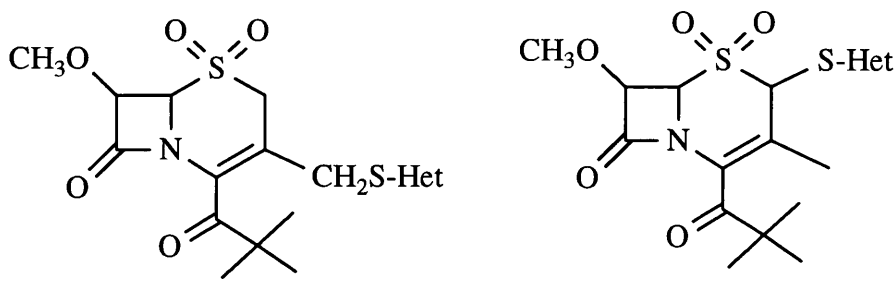
A recent paper[106] from Italian chemists has described novel cephem-4-ketones which have important human Leucocyte Elastase (HLE) inhibiting properties. Cephalosporanic acids (**234; X=OH**) were converted into the corresponding acid chlorides (**234; X=Cl**) by oxalyl chloride in DMF/THF at 0°C which on treatment with various Grignard reagents and copper (I) iodide in THF at -40 to -70°C were transformed into the analogous ketones (**235a-d**).



(**234**)

(**235**) a; R = Bu^t
b; R = Et
c; R = Ph
d; R = CH_2Ph

Furthermore the *t*-butyl ketone substituted at either C-2 or C-3 with thioheterocyclic derivatives eg (**236**) and (**237**) along with the unsubstituted ketones (**235a-d**) all proved to be potent HLE inhibitors.



(**236**)

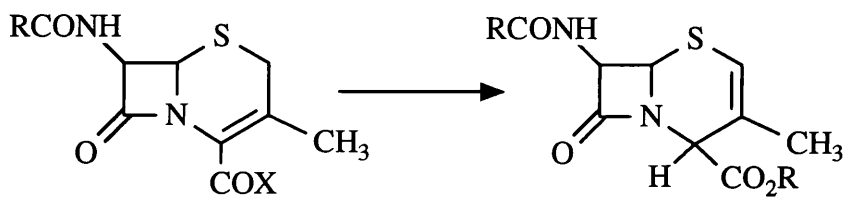
(**237**)

1.5.4 Ester Formation and Deprotection

The most common C-4 carboxyl reaction is esterification in which simple esters have been easily prepared (eg methyl) with the analogous diazoalkanes[107]. However removal of these ester groups has caused such difficulty that in depth research, into more suitable and easily removed protecting groups

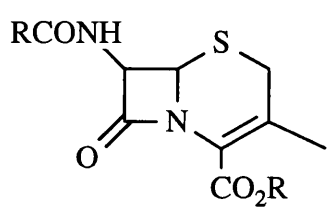
with reagents that are not detrimental to the β-lactam ring, has occurred. Ester formation must also take place under conditions that prevent Δ2/Δ3 isomerisation.

Murphy and Koehler[108] investigated the preparation of cephem esters from deacetoxymethyl cephalosporins *via* a reactive ketene. Using oxalyl chloride (other chlorides such as thionyl chloride and phosphorus pentachloride were less effective) in an inert solvent and DMF as a catalyst, **(238; X=OH)** was converted into the acid chloride **(238; X=Cl)**. Esterification conditions varied according to the alcohol employed. Tertiary alcohols were used in excess at ice-bath temperatures, primary and secondary were used at lower temperatures with approximately equivalent amounts resulting in the cephem esters **(239)** with a Δ2-double bond.



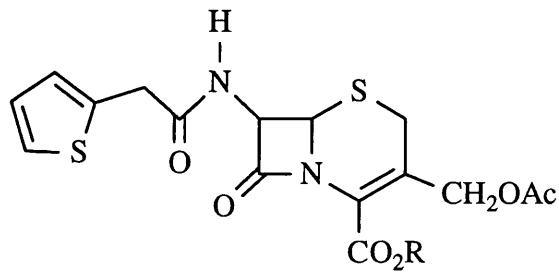
(238) **(239)**

A high yielding esterification process with possible industrial applications has also been reported[109]. Cephalosporins **(238; X=OH)** were converted into their corresponding alkyl esters **(240)** by reaction of the sodium or potassium salts with alkyl bromides in acetonitrile using crown ethers as phase-transfer catalysts. The esterification generally proceeds within 24 to 48 hr at room temperature and the crude products **(240)** exhibit sharp melting points close to those of the purified compounds.



(240) R = PNB and Bz

A preparation which avoids the Δ3 to Δ2 isomerisation was reported by Chicago University chemists[110]. Several esters **(241)** of cephalothin **(11)** were produced by suspending the cephem acid **(241; R=H)**, sodium carbonate and an alkyl bromide or iodide at room temperature in a DMF/*p*-dioxane mixture. The yields of the pure ceph-3-em esters were reported to range from 48 to 61%.



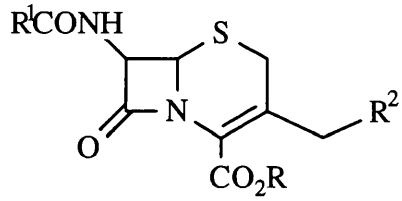
(241)

R = PNB, Bz, Me, allyl and 2-oxo-1-propyl

Esterification of cephalothin was also investigated by Japanese chemists[111] using various alcohols in the presence of 2,4,6-tripyridinio-1,3,5-triazine trichloride which was prepared *in situ* in CH_2Cl_2 from cyanuric chloride reacted with 3 molar equivalents of pyridine. Good to excellent yields (48-92%) were obtained of **(241)**.

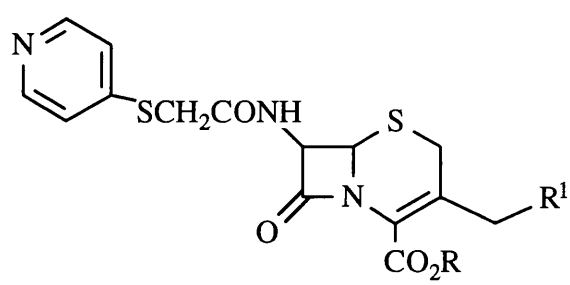
Protecting groups have been removed by a variety of methods eg TFA[112], formic acid[113], thiols[114] or a mixture of Lewis acids with anisole[115], but large quantities of acid are required to complete the reaction in most of these cases and hence limits the number of protecting groups available. This problem has been investigated and overcome by employing phenol as the reaction medium[116]. Cephalosporin esters **(242a-e)** were deprotected by gentle heating (45-60°C) in phenol in the presence of various acids (TFA, HCl, H_2SO_4, *p*-TsOH and $KHSO_4$). Ester cleavage occurred in high yields of 86 to 96%. Reaction also proceeded in the absence of the acid catalysts ie **(242a)** was converted in a 30% yield to its corresponding acid at 45°C and at 60°C conversion was accomplished in 96% yield. However these conditions do not apply to all esters as **(242c)** and **(242e)**

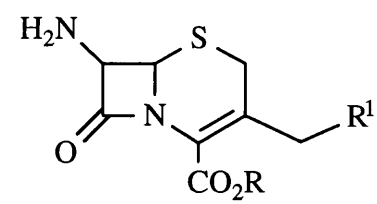
were recovered intact after heating at 45°C in phenol for 1 hr in the presence of TFA.



(242) a; R = PNB
b; R = $CHPh_2$
c; R = CH_2Ph
d; R = Bu^t
e; R = PMB

A mild and convenient method for deblocking of benzhydryl esters only, has been reported[112] without decomposition of the β-lactam ring. Cephalexin benzhydryl ester **(242b; R^1=COCH(NH_2)Ph)** was cleaved to its corresponding acid in a 70% yield by warming in 98-100% formic acid at 40-45°C for 30 min. To analyse the scope of the reaction, benzhydryl esters **(243a)** and **(244a)** were similiarly converted to the analogous cephalosporanic acids **(243b)** and **(244b)** in 84% and 90% yields respectively.





(243) a; R = $CHPh_2$
b; R = H

(244) a; R = $CHPh_2$
b; R = H

R^1 = CH_3 or OAc

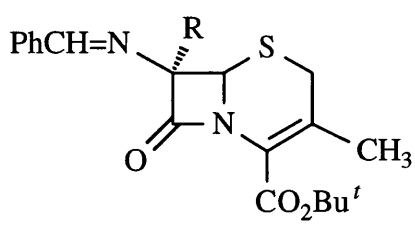
1.6 Reactions at C-7

1.6.1 7α-Methoxycephalosporins

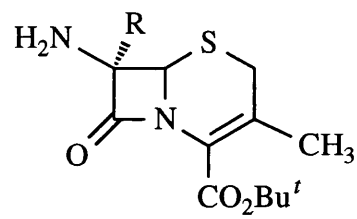
Approximately 17 years after cephalosporin C was discovered, Lilly[117] and Merck[118] laboratories discovered the production of 7α-methoxycephalosporins from *streptomyces.* The incorporation of a 7α-methoxy substituent into the cephalosporin nucleus induces stability towards β-lactamase hydrolysis without

affecting the anti-bacterial activity and hence created intensive research into the preparation of semisynthetic cephamycin derivatives. Two main routes exist for introducing the methoxy group into the 7α-position, either by nucleophilic attack on a range of 7-imino intermediates[119] or reaction of the 7-carbanion with electrophiles[120].

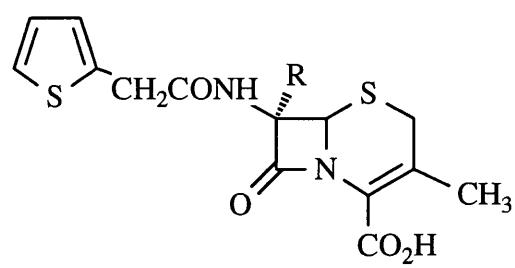
Jen *et al*[121] using the latter method, prepared the 7α-methoxycephalosporanic acid **(247)** which displayed poor anti-bacterial activity. Treatment of 7-benzylideneaminocephalosporin ester **(245; R=H)** with $MeSSO_2Me$ and NaH afforded **(245; R=SMe)** in a 60% yield. Addition of HCl in acetone furnished **(246; R=SMe)** which in the presence of pyridine with $HgCl_2$ in CH_3OH/DMF was converted to the crystalline 7-methoxy derivative **(246; R=OMe)** in an 80% yield. Acylation with 2-thienylacetyl chloride followed by de-esterification gave a 67% yield of **(247)**. Similiar[122] 7α-methoxycephem was



(245)



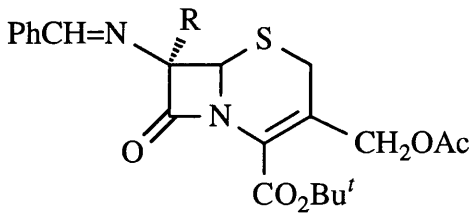
(246)



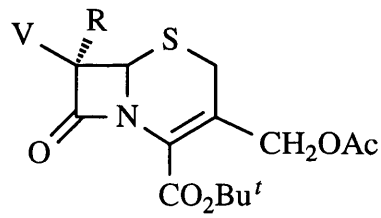
(247)

prepared by treatment of Schiff base **(248; R=H)** with potassium *tert*-butoxide followed by $CH_3SO_2SCH_3$ to give **(248; R=SMe)** in 50% yield. Acylation with phenoxyacetyl chloride in CH_2Cl_2 resulted in **(249; R=SMe)** which was reacted with methanol and mercuric acetate to produce the 7α-methoxy derivative **(249; R=OMe)** in 47%. Deprotection by standard methods resulted in the free acid

(250) which was inactive.



(248)



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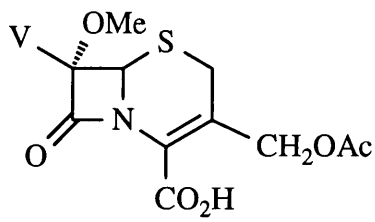
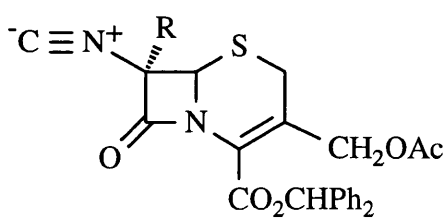
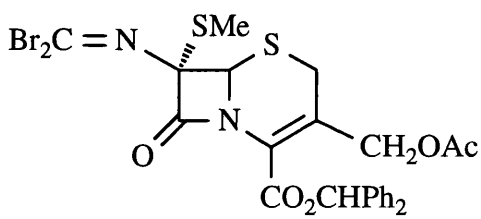


(250)

Jung and colleagues[123] synthesised 7α-methoxy analogues with novel aminobenzimidazole and aminoimidazoline heterocycles at C-7. Treatment of 7-isocyanocephalosporin **(251; R=H)** under argon with methylsulphenyl O-methyl thiocarbonate in the presence of cuprous oxide (Cu_2O) afforded **(251; R=SMe)** in a yield of 50%. Addition of Br_2 in CCl_4 dropwise over a 30 min period to a toluene solution of **(251; R=SMe)** afforded compound **(252)**. Utilizing a similar procedure

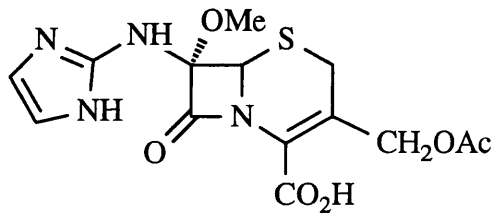


(251)

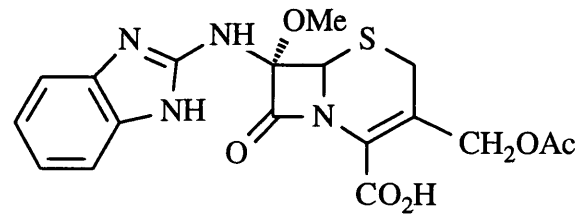


(252)

as previously reported[121] ie SMe-OMe exchange and deprotection cephems, **(253)** and **(254)** were prepared. However neither compound displayed biological activity.

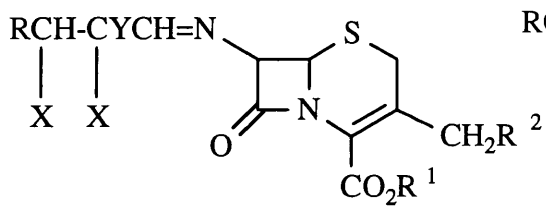


(253)

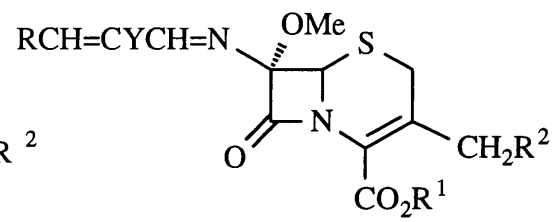


(254)

A 'one-pot' method for preparation of cephamycin derivatives was published by Atsumi *et al*[124]. Using excess methanol with borax ($Na_2B_4O_7.10H_2O$) as an acid scavenger, the Schiff base (**255**) was converted into the 7α-methoxycephem (**256**). High yields of between 70 and 95% were achieved.



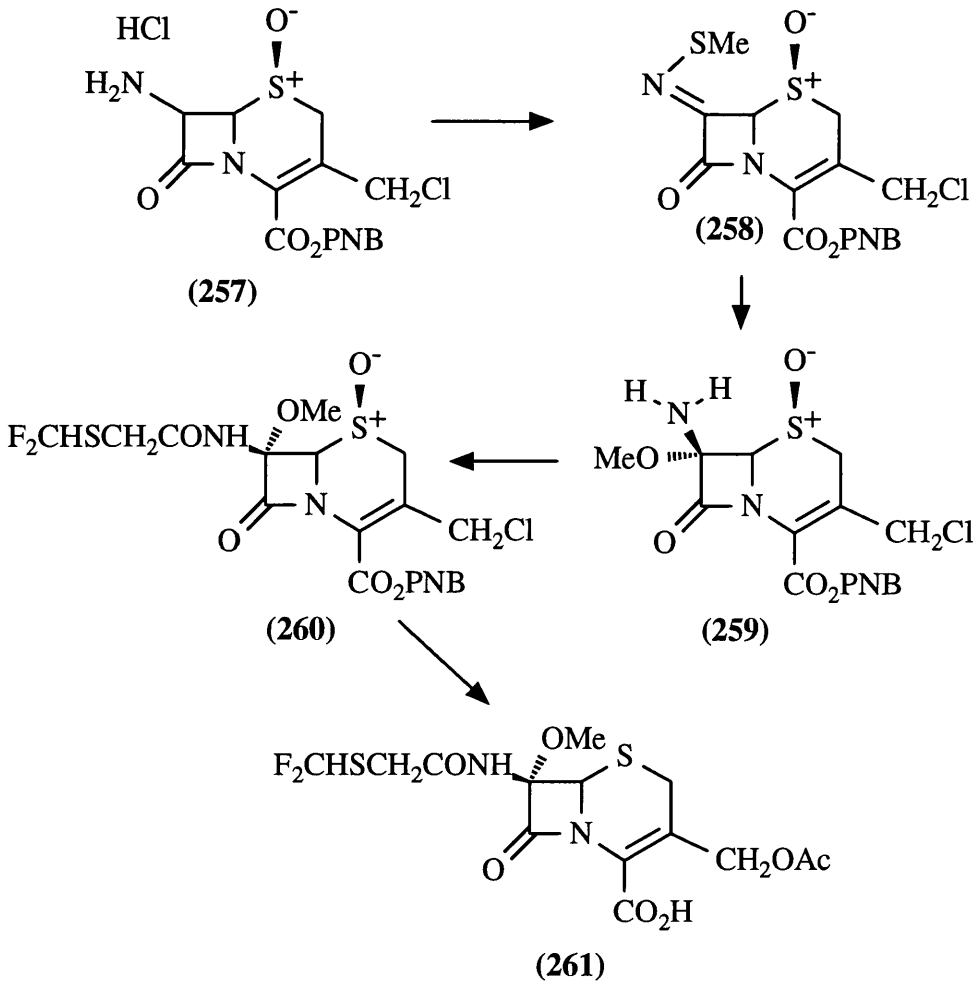
(255)



(256)

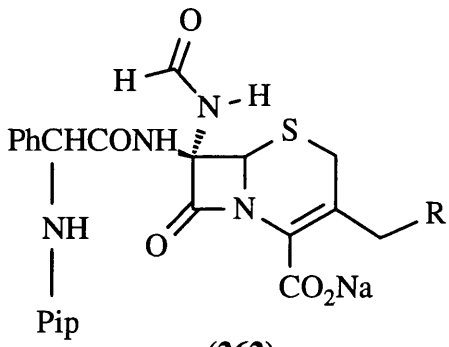
X = Y = Cl or Br

To overcome the previous problem of low yielding reactions, a new stereoselective methoxylation which is viable on industrial scale was described by Shionogi chemists[125]. In accordance with a procedure determined by the Squibb group[126], ceph-3-em (**257**) reacted with 3 molar equivalents of methylsulphenyl chloride and resulted in a 93% yield of (**258**). Conversion to the 7α-methoxy adduct (**259**) occurred with a MeOH-THF mixture and 2 molar equivalents of HCl at -20°C . Acylation of (**259**) with difluoromethylthioacetyl chloride gave the desired 7α-methoxy derivative (**260**) in 62.3% yield which was reduced to the sulphide in a yield of 96%, substituted at C-3 and deprotected to give (**261**). The presence of the 1β-sulphoxide creates hydrogen bonding with the 7β-amino group, thus preventing nucleophilic attack on the β-face, and hence leading to the predominant formation of the stable 7α-methoxycephem in high yield.

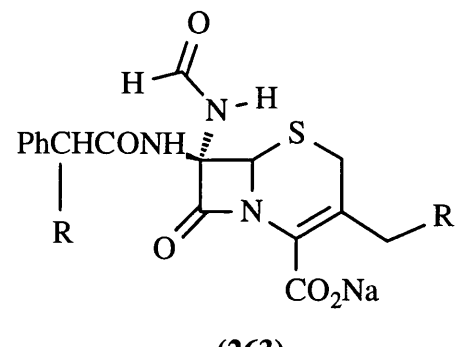


1.6.2 7α-Formamidocephalosporins

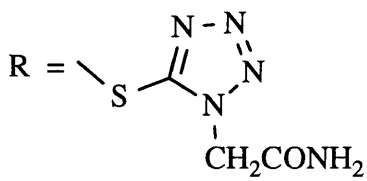
Until recently the synthesis of penicillins and cephalosporins bearing substituents other than methoxy at the 6α- and 7α-positions produced compounds which showed reduced activity compared to their unsubstituted analogues. However although naturally occuring 7α-formamidocephalosporins have low bacterial activity[127] there are semisynthetic derivatives which exhibit outstanding broad-spectrum activity. Varying substituents on the 3-methyl group eg **(262)** and 7β-amido side-chain eg **(263)** has produced 7α-amidocephems which have displayed an excellent combination of antibacterial activity and β-lactamase stability[128].

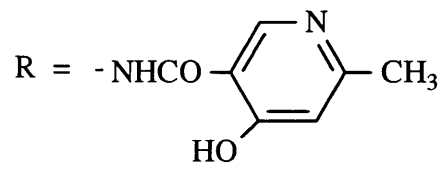


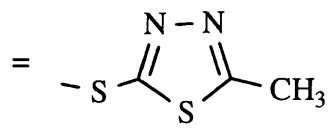
(262)

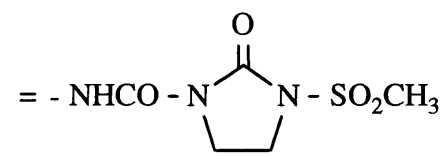


(263)

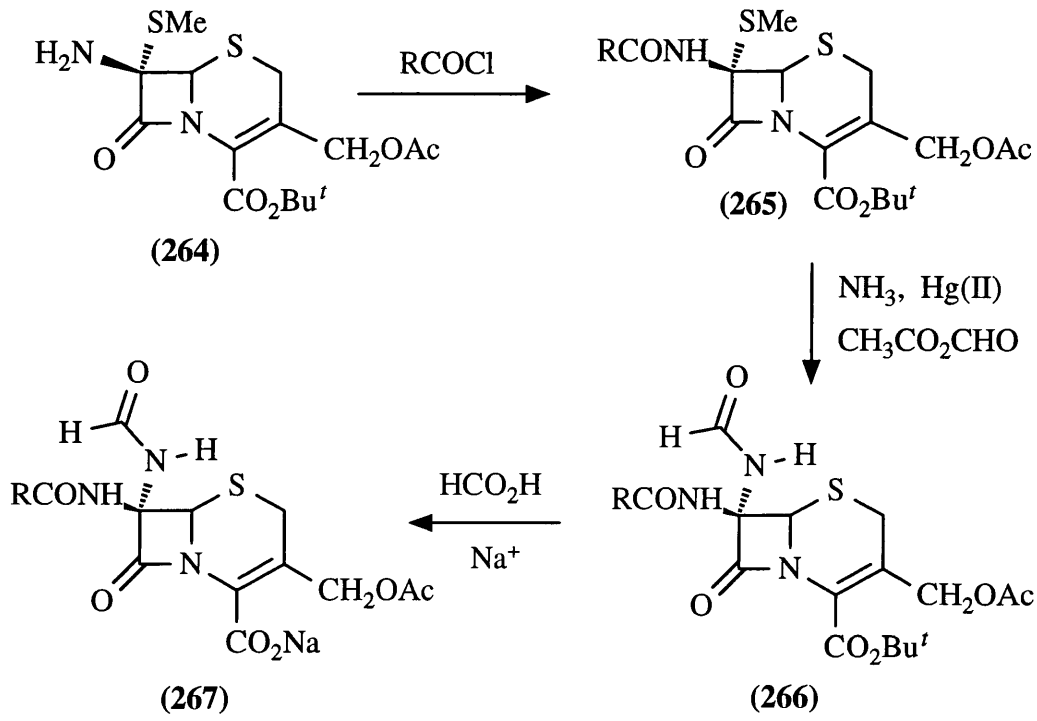




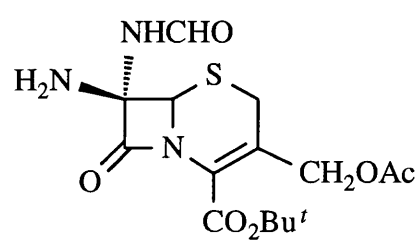




= OAc



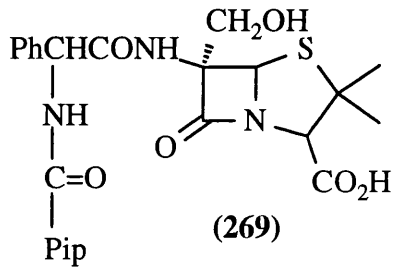
procedure. However an alternative route is also described[129] which readily provides the highly crystalline 7β-amino-7α-formamido analogue (**268**), a more versatile intermediate. Employing (2,2,2-trichloroethoxy)carbonyl chloride resulted in acylation and protection of the 7β-amine group affording (**265; R=Cl_3CCH_2OCO**). Amination with Hg (II), followed by formylation with acetic formic anhydride gave (**267; R=Cl_3CCH_2OCO**) which was reduced with Zn and HCl to give (**268**). Acylation of the amine (**268**) with acid chlorides required shorter reaction times and resulted in a wider range of 7α-formamidocephalosporins (**266**) with improved yields.



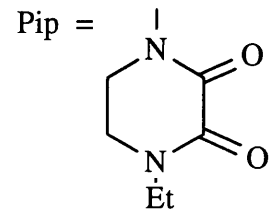
(**268**)

1.6.3 Other 7α-substituents

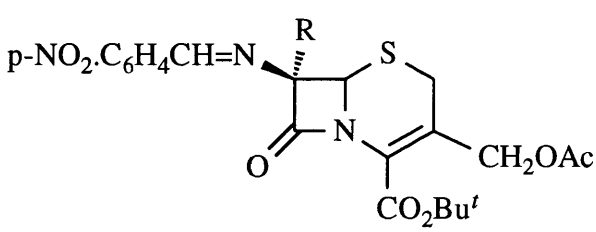
Apart from 7-methoxy and 7-formamido, 7α-hydroxymethyl substituents also display some biological activity. After discovering that certain penicillins with 6α-hydroxymethyl groups, as exemplified by **(269)**, exhibit anti-bacterial activity, Dixon *et al*[130], investigated the 7α-hydroxymethyl-cephalosporins. Preparation involved treatment of the Schiff's base **(270a)** with



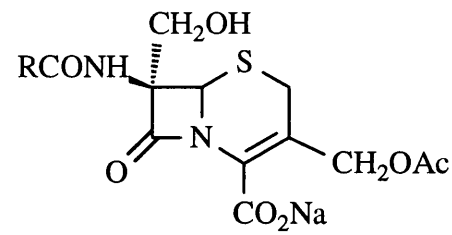
(269)



anhydrous potassium carbonate in DMF at -22°C followed by quenching with gaseous formaldehyde forming the alcohol **(270b)**. Acylation and deprotection afforded the sodium salt **(271)**.



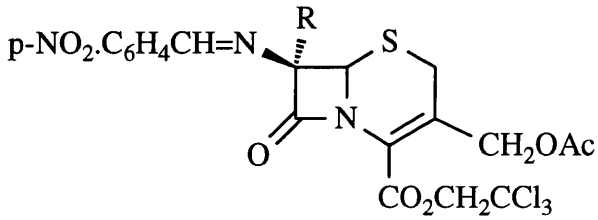
(270) a; R = H
b; R = CH_2OH



(271)

Similiarly, by treating the Schiff base **(272a)** in a THF-DMF mixture with a 10mmol THF solution of lithium diisopropylamide at -78°C followed by trichloroethyl chloroformate resulted in the acylated cephem **(272b)** in 90% yield[131]. Reaction of **(272b)** with amino-oxyacetic acid hemihydrochloride in THF-MeOH solution afforded **(273a)** in 34% yield which when treated at -45°C with an acid chloride in CH_2Cl_2-pyridine resulted in the amide **(273b)**.

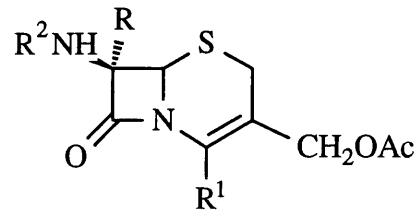
De-esterification using Zn/AcOH in DMF gave the diacid (**273c**) in good yield. Various 7-substituted ceph-3-ems were produced using different acyl halides and although all exhibited antibacterial activity, it was less so than the corresponding unsubstituted parent compound.



(**272**)

a; R = H

b; R = $CO_2CH_2CCl_3$



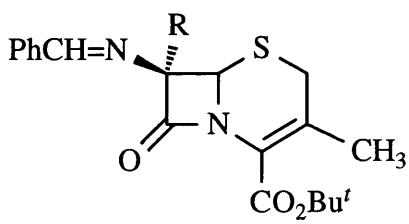
(**273**)

a; R = R^1 = $CO_2CH_2CCl_3$
R^2 = H

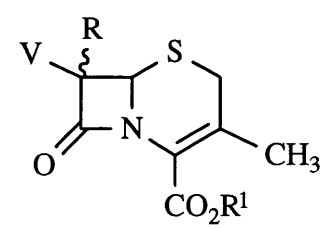
b; R = R^1 = $CO_2CH_2CCl_3$
R^2 = $COCH_2.SC_4H_4$

c; R = R^1 = CO_2H
R^2 = $COCH_2.SC_4H_4$

Synthesis of the 7α- (and 7β-) methylcephalosporins has also been reported[132] but both isomers were inactive. The 7α-methylcephem (**274; R=Me**) was produced by alkylation of the cephalosporanic ester (**274; R=H**) using methyl iodide and sodium hydride in dimethoxyethane at 0°C. Acylation with phenoxyacetyl chloride in aqueous chloroform afforded the *tert*-butyl esters (**275; R^1=Bu^t**) in a yield of 85% which were deprotected by standard procedures to give the acids (**275; R^1=H**).



(**274**)

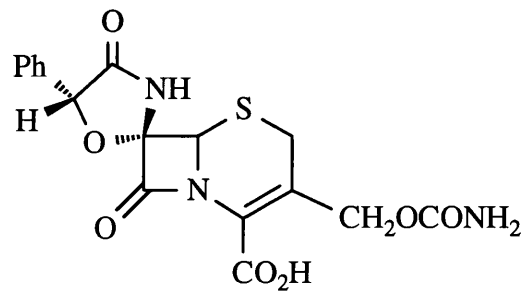


(**275**)

A similar sequence of reactions was accomplished with the penicillin series and the 6-methyl analogues were also inactive.

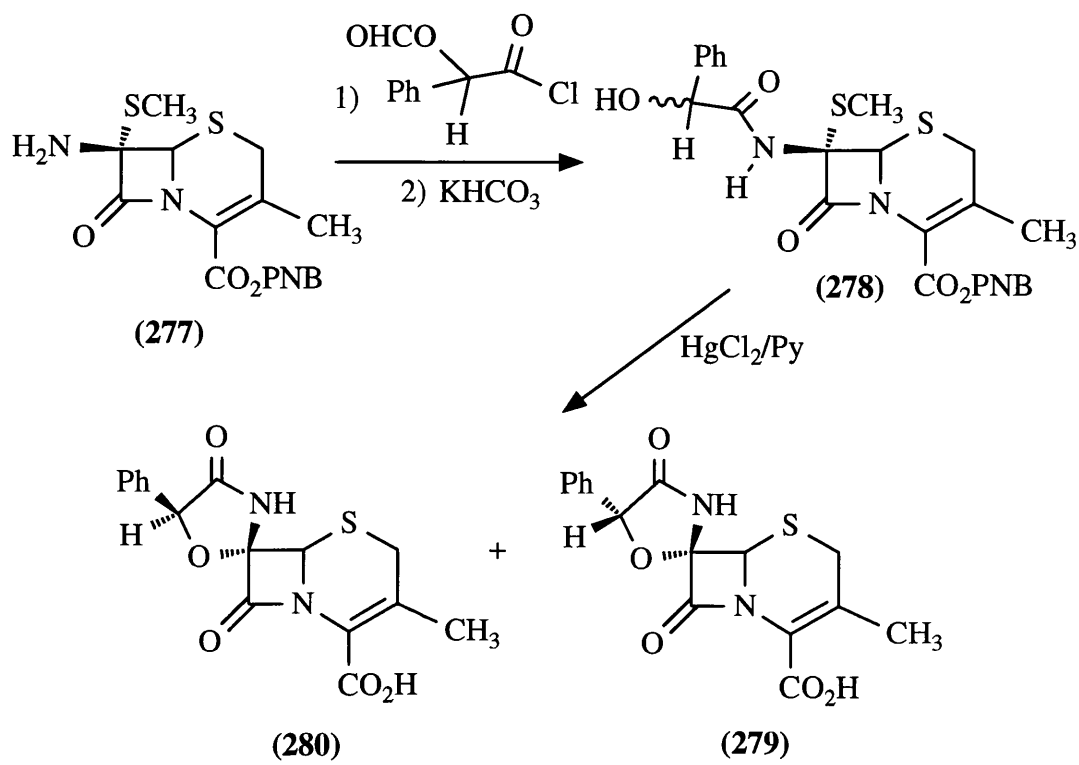
1.6.4 7-Spirocyclic-Cephalosporins

Koppel and Koehler[133] produced a cephamycin analogue **(276)** which contained a 7-spirocyclic ring system and displayed antibiotic activity hence interest by Sammes *et al*[134] in the preparation of similar 7-substituted cephalosporins. Treatment of the 7α-thiomethylcephem **(277)** with

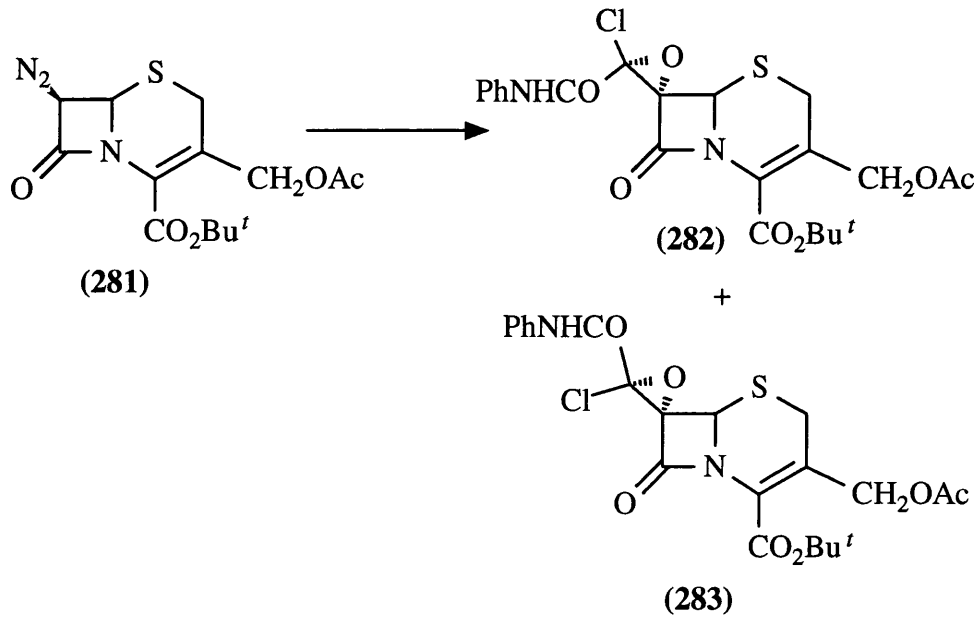


(276)

formyloxy(phenyl)acetyl chloride, followed by deformylation using potassium hydrogen carbonate in MeOH-acetone resulted in **(278)**. Cyclisation was induced with mercuric chloride and pyridine in DMF furnishing spiro-compounds **(279)** and **(280)** which were de-esterified to the corresponding acids of which only the (S)-isomer **(279)** exhibited some biological activity.

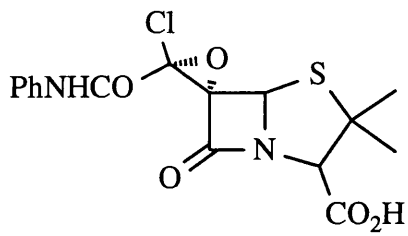


From an unpredictable reaction between 7-diazocephalosporin (**281**) and oxalyl chloride, the spiro-cyclic cephalosporin (**282**) and its isomer (**283**) were produced[135]. However after de-esterification, the minor adduct (**282**) displayed



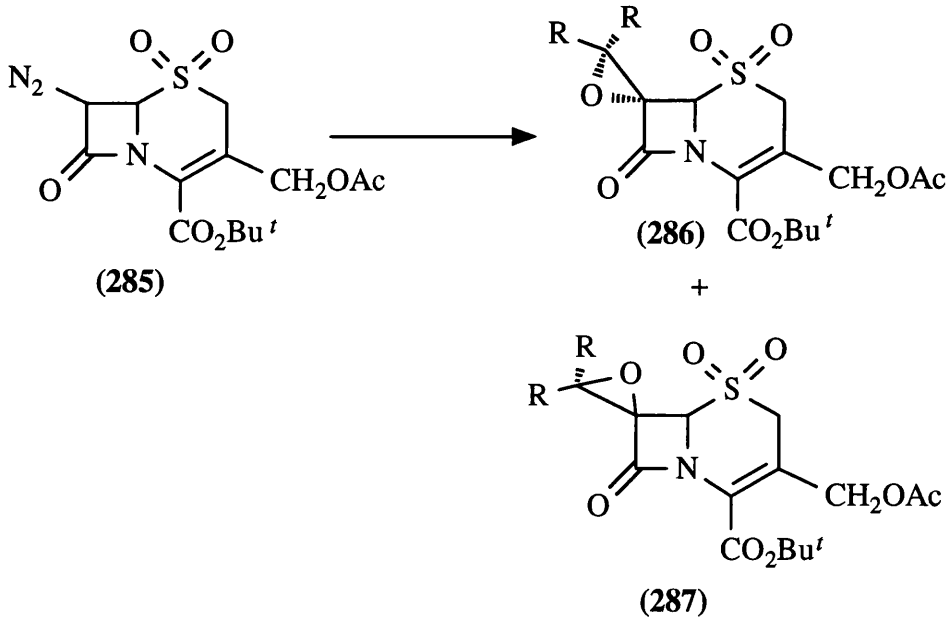
(**281**) (**282**) (**283**)

only moderate activity. Similiar reactions investigated in the penicillin family resulted in the spiroepoxide analogues (**284**) that exhibited good inhibitory properties towards β-lactamase hydrolysis.



(**284**)

The 7-diazocephalosporins have been extensively used in the preparation of 7α-substituted compounds. However SynPhar laboratory[136] reacted the 7-diazosulphones (**285**) with various aldehydes and observed that if a small excess of aldehyde in CH_2Cl_2 was used with a boron trifluoride etherate catalyst, then the major product obtained was the 7α-spiroepoxycephem (**286**) with the minor isomer (**287**).

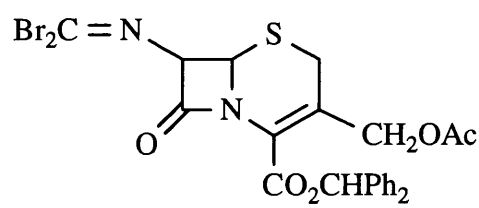


(285) (286) (287)

R = either H or R from RCHO

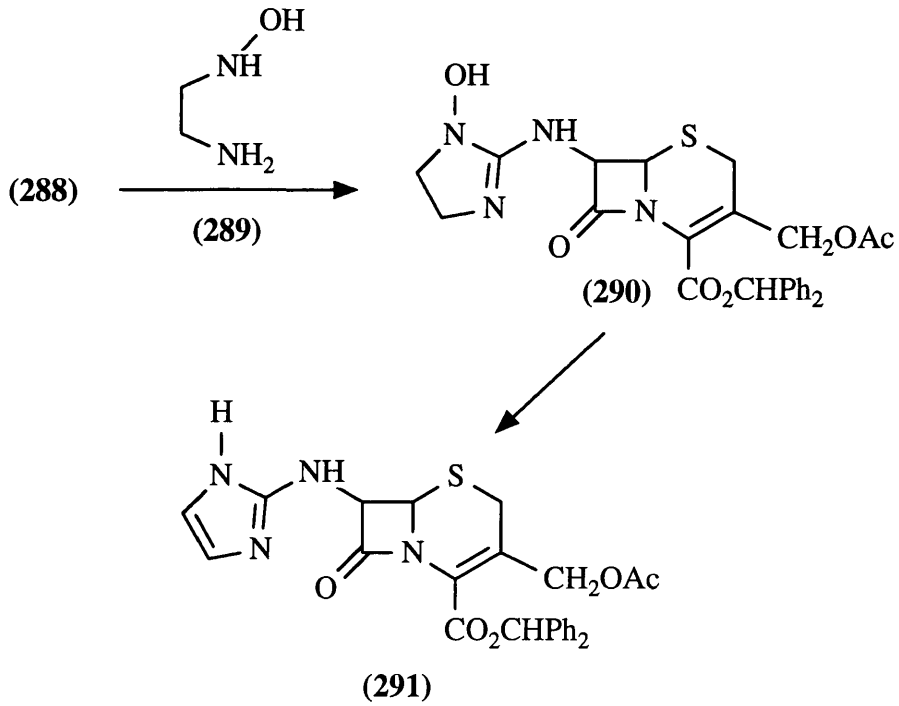
1.6.6 Reactions at N-7

Jung *et al*[137&138] describe the preparation of ceph-3-ems with amino heterocyclic side chains at C-7 from the precursor (**288**). This dihalide group is



(288)

more electrophilic than the β-lactam ring and thus is the location for incoming nucleophilic attack. Compound (**288**) was reacted with several dinucleophiles, firstly condensing (**289**) with the dibromoisonitrile (**288**) in THF at -40°C gave (**290**). Further reaction with 2-fluoro-N-methyl-pyridinium tosylate in CH_2Cl_2 with triethylamine resulted in the desired product (**291**).



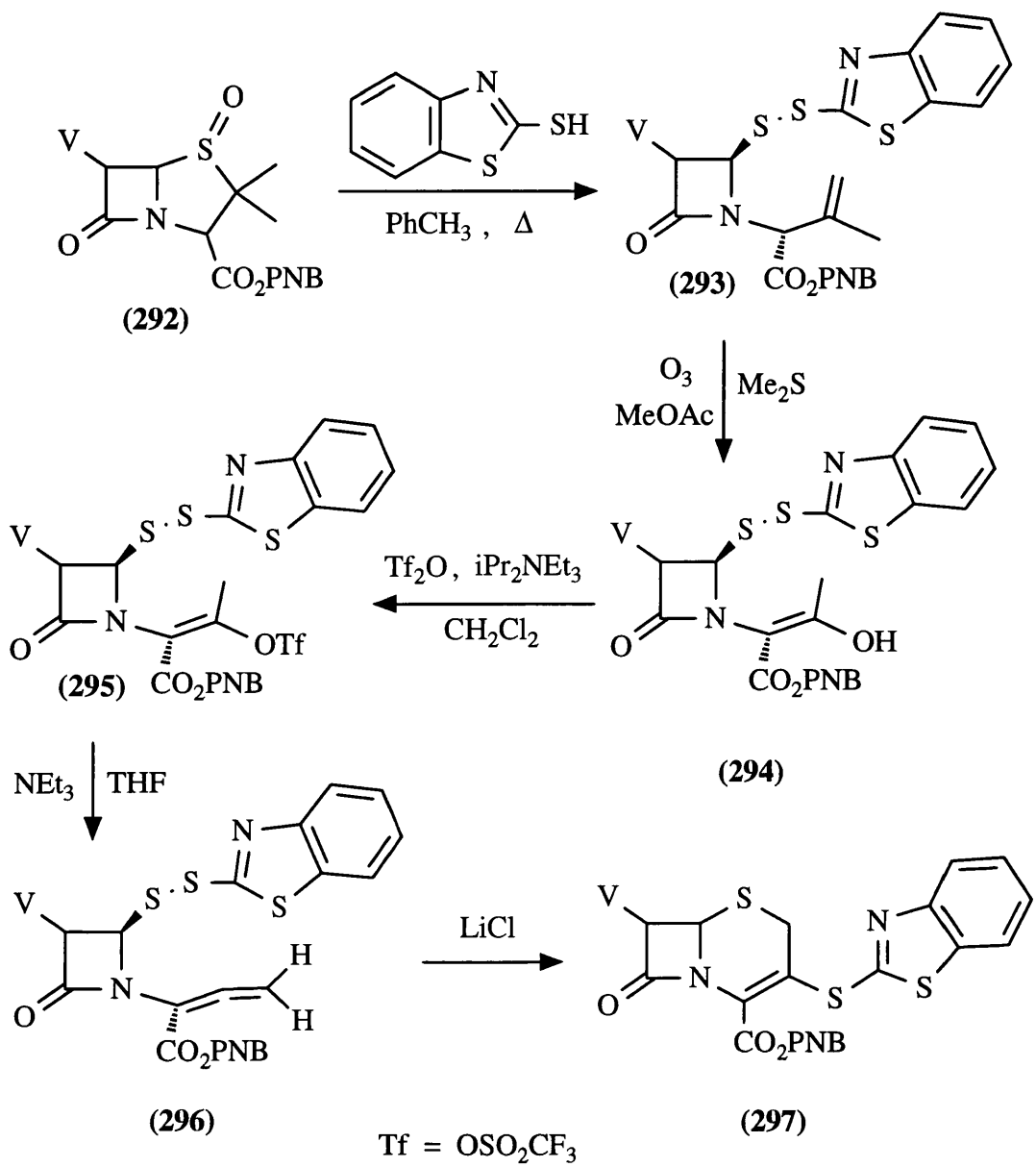
1.7 Miscellaneous Cephalosporin Reactions

1.7.1 Conversion of Penicillins to Cephalosporins

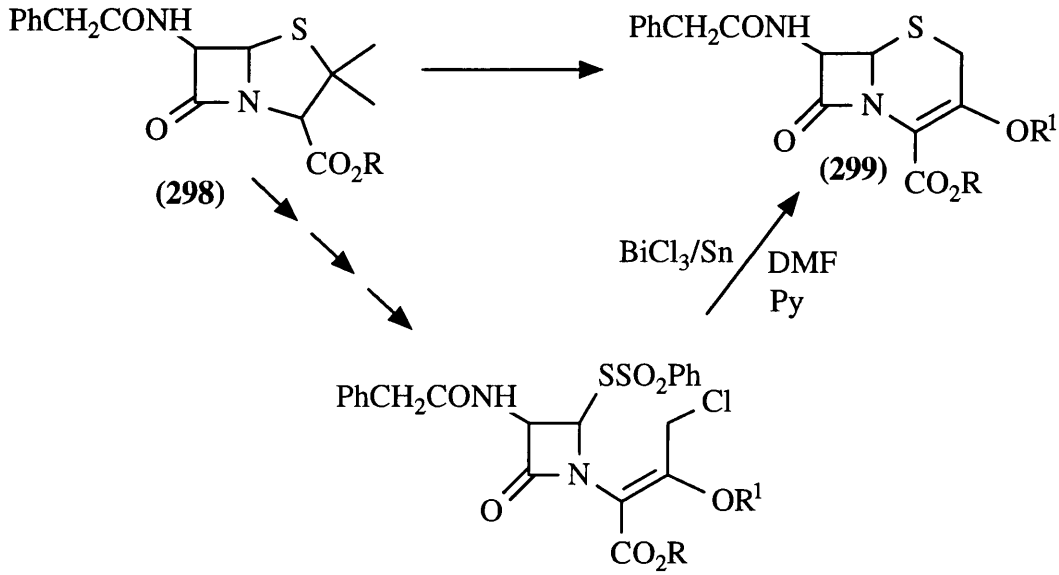
The possibility that cephalosporin C was a metabolic transformation product of penicillin N and the ready availability of the penicillin nucleus 6-APA provided the additional impetus for the study of penicillin-cephalosporin conversion.

Morin's general approach[139] to the problem was to activate the sulphur atom either by alkylation or oxidation followed by the cleavage of the C-2 sulphur bond; incorporation of a double bond and reclosure of the ring to form a cephalosporin. In a later paper, Morin[19] studied the reactions of penicillin sulphoxide **(8)** and discovered that refluxing in acetic anhydride afforded two substances, the desired cephalosporin **(9)** and **(10)**. Morin's rearrangement was a classic development but led only to C-3 methyl derivatives. However a publication[140] extending his work developed an efficient procedure for the synthesis of 3-norcephalosporins bearing C-3 sulphur moeties from an allenic

intermediate. Penicillin V sulphoxide (**292**) was converted to (**293**) in a high yield of 85% by cleavage of the thiazolidine ring followed by treatment with 2-mercaptobenzothiazole in refluxing toluene. Reaction of (**293**) with ozone in acetic acid at -78^{O}C furnished the enol (**294**) which in the presence of Tf_2O and *i*-Pr_2NEt in CH_2Cl_2 afforded the triflate (**295**) in 87% yield. Under mild elimination conditions, (**295**) was converted in 100% yield into the allene (**296**) which was further reacted *in situ* with lithium chloride in THF to promote cyclisation to ceph-3-em (**297**).



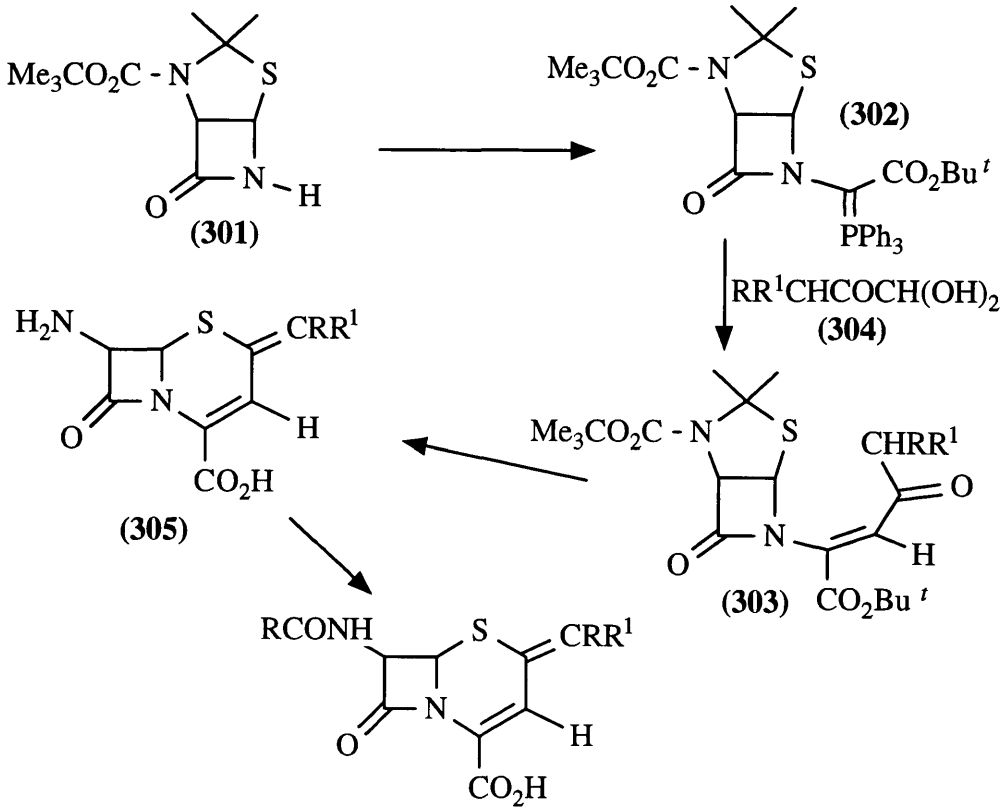
The greater number of penicillin-cephalosporin transformations involve the oxidised state however a recent report by Japanese workers[141] details a direct conversion from the sulphide of penicillin G **(298)** to 3-hydroxycephems **(299)** involving the critical cyclisation of **(300)** with the novel $BiCl_3/Sn$ or $TiCl_4/Sn$ bimetal redox system.



(300)

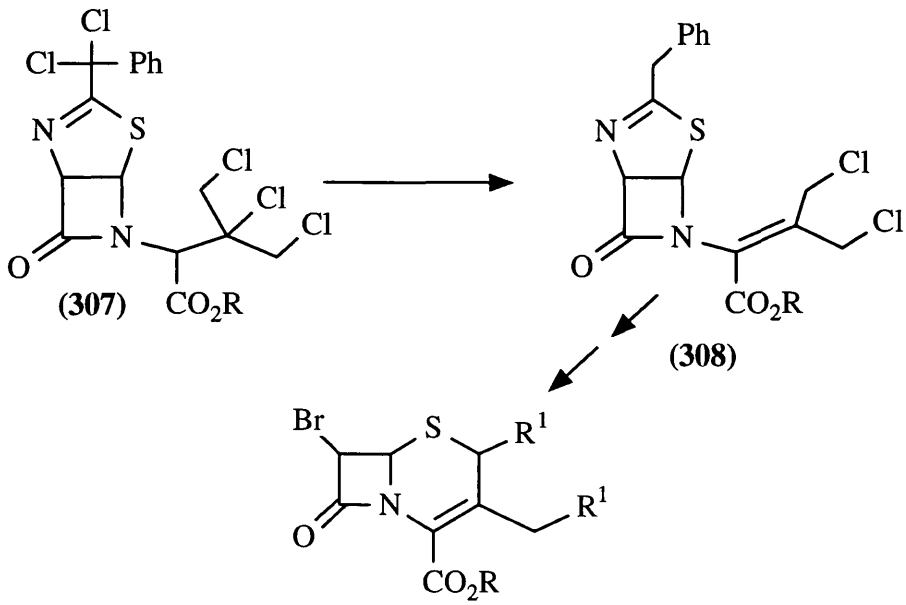
R^1 = alkyl

Thiazolidine-azetidinones **(301)** which can easily be derived from penicillins play an important part in the chemical synthesis of cephalosporins from penicillins. Woodward *et al*[142] prepared the phosphorane **(302)** from **(301)** which was converted into the fumaroid isomer **(303)** by heating in toluene or dioxane with an hydrated α-ketoaldehyde **(304)**. Deprotection with trifluoroacetic acid followed by an intramolecular condensation and a simultaneous conversion into the free acid resulted in **(305)** which was acylated to the biologically active cephalosporin **(306)**.



(306)

Similiarly pentachloride **(307)** undergoes intramolecular cyclisation into **(309)** *via* dichloride intermediate **(308)**[143].

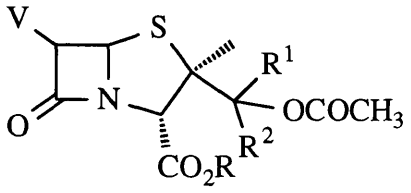


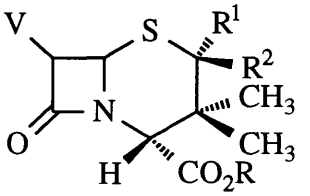
(309)

1.7.2 Conversions of Cephalosporins to Penicillins

As a consequence of the economic route from penicillins to cephalosporins, chemists have almost exclusively concentrated their research in this area and few publications exist on the reverse transformation ie cephalosporins into penicillins.

One report[144] investigated this latter conversion in an attempt to prepare penicillin derivatives more complicated than those from natural origin. The penam structures **(310a)** and **(310b)** were obtained from the mixture of **(311a)** and **(311b)** with silver acetate in glacial acetic acid at 100°C.

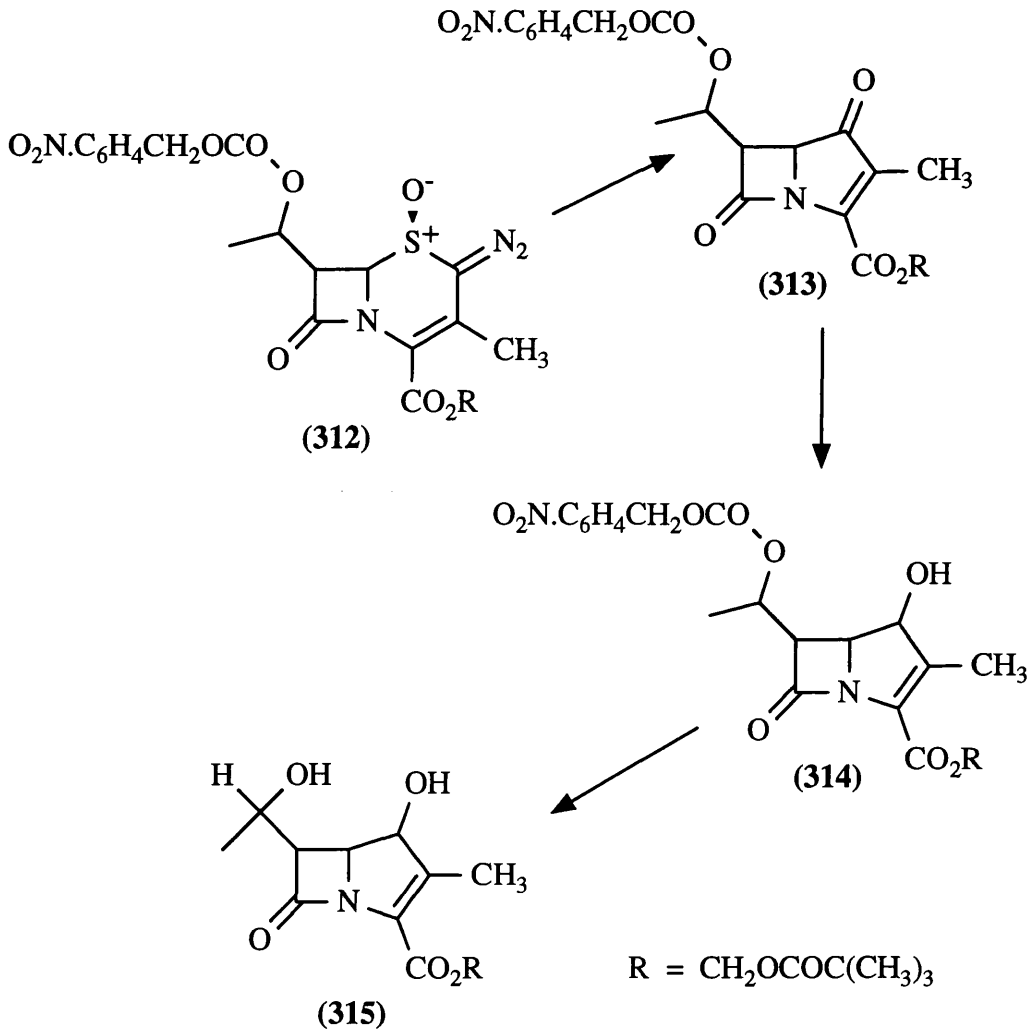




(310) a; $R^1 = OCH_3$, $R^2 = H$
b; $R^1 = H$, $R^2 = OCH_3$

(311) a; $R^1 = OCH_3$, $R^2 = H$
b; $R^1 = H$, $R^2 = OCH_3$

Another route for deriving the penicillin nucleus and widely researched by the Pfizer laboratories[145&146] was photolysis. Subjecting the diazoceph-3-em **(312)** dissolved in CH_2Cl_2 in a pyrex vessel to a 275 watt sun lamp furnished **(313)** which was promptly reduced to the alcohol **(314)** with tetrabutylammonium borohydride. Hydrogenation with a Pd/C catalyst afforded the carbapenem **(315)** which exibited a broad spectrum of activity.



1.7.3 Tricyclics and Tetracyclics

Numerous diverse tricyclic derivatives have been described most of which have been synthesised by one of the following three possible pathways:-

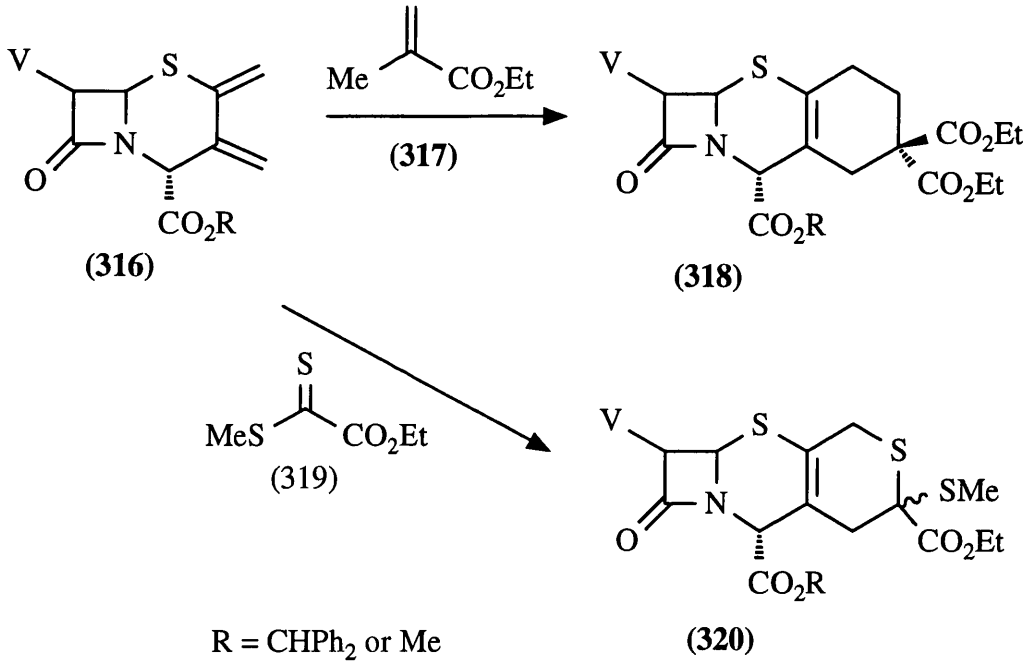
a) 1,3-cycloaddition

b) Wittig condensation

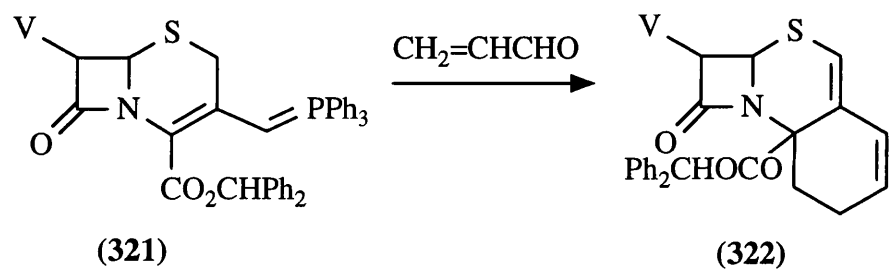
c) free-radical cyclisation

Via a Diels-Alder cycloaddition reaction, **(316)** reacts with **(317)** in toluene at 90-95°C for 12 hr affording **(318)** in 12% yield. Cepham **(316)** also reacts with **(319)** in dry benzene at room temperature for 12 hr to give a

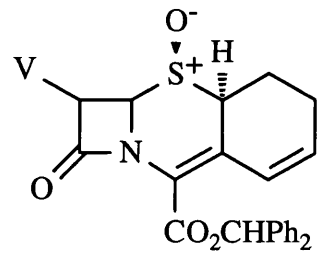
diastereomeric mixture of **(320)** in 32% yield [147].



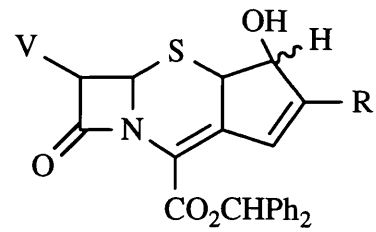
Alternatively cephalosporins bearing a phosphorane at C-3, as in **(321)**, undergo reaction with an aldehyde followed by an internal Wittig condensation affording the tricyclic cephem **(322)**[148].



Similiarly the corresponding sulphoxide of **(321)** reacts to give the tricyclic fused at the 2,3-positions **(323)**. These authors continued their investigations[148] into C-3 phosphorane cephalosporins by reaction of the ylide **(321)** with methyl glyoxal at -20°C in DMF which resulted in the tricyclic ceph-3-em **(324a)** in a 46% yield. At 40°C, the same reaction gave almost exclusive formation of **(324a)** in 57% and at room temperature, reaction of **(321)** with glyoxal afforded the tricyclic **(324b)** in 50% yield.

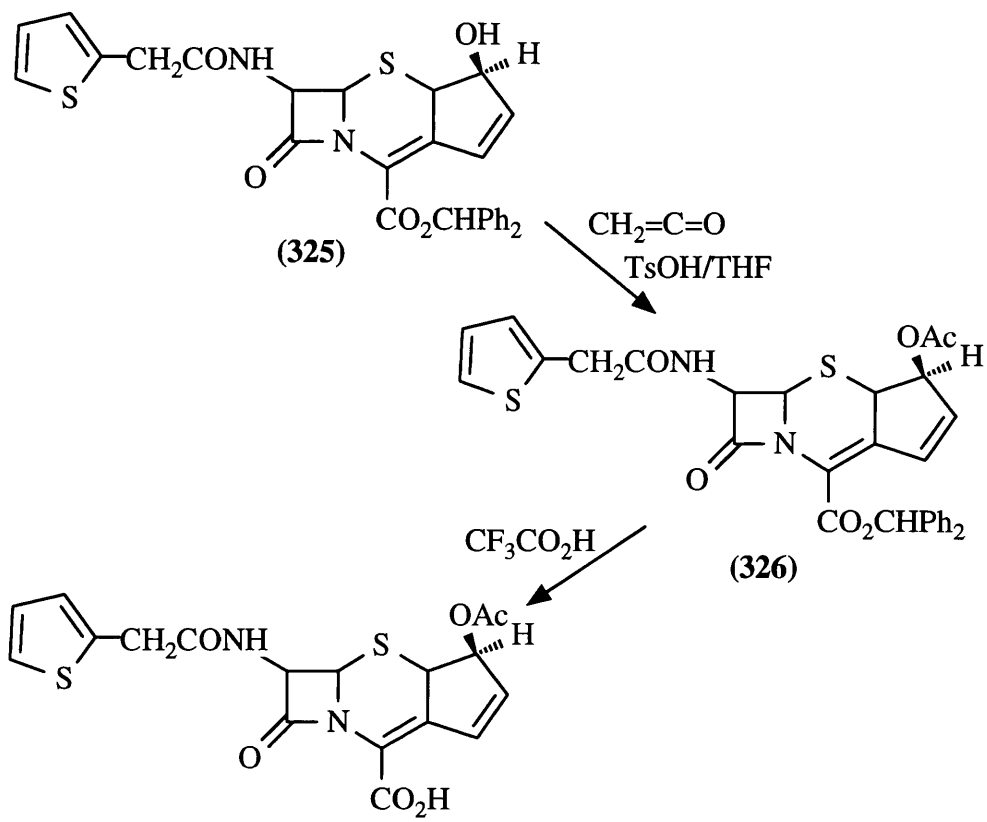


(323)



(324) a; R = CH_3
b; R = H

To test biological activity cephalosporin (**325**) was prepared and converted to (**326**) *via* acetylation with ketene. Treatment of (**326**) with trifluoroacetic acid resulted in a single isomer (**327**) which exhibited activity against gram-positive organisms but no significant activity against gram-negative organisms.

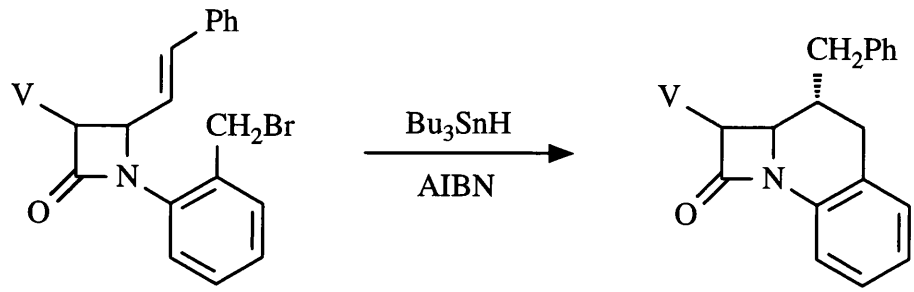


(325)

(326)

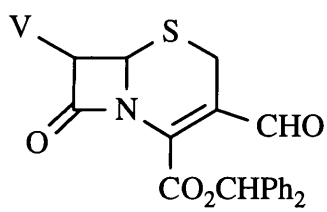
(327)

In the presence of Bu_3SnH and azobisisobutyronitrile (AIBN)[149], the tricyclic (**329**) was furnished from (**328**) by free radical cyclisation.



(328) **(329)**

An interesting conclusion to this section is the preparation of **(331)**, the first novel ring system which comprises the structural unit of a cephem, penicillin and γ-lactam in the same molecule[150]. These were produced by a Hungarian research group *via* a condensation reaction of the ceph-3-em **(330)** with D-penicillamine in methanol:chloroform.



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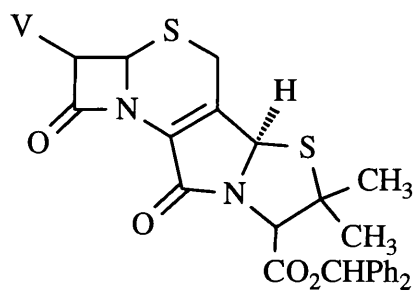
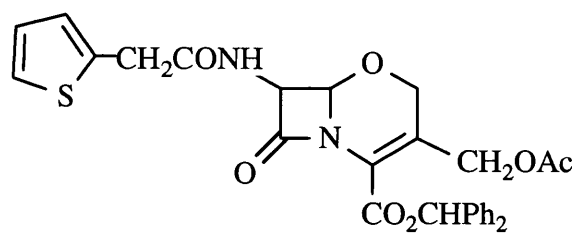


(331)

1.7.4 Nuclear analogues

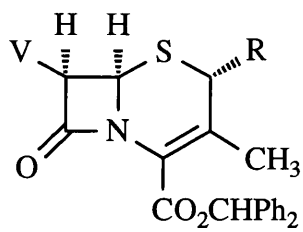
It was first demonstrated in 1974[151] that the sulphur atom was not a requisite atom for the biological activity of cephalosporins and that the 1-oxacephalosporins **(332)** had analogous activity to cephalothin **(11)**. Since those findings 1-oxacephems along with 1-carbacephems have been intensively researched.



(332)

1.7.4.1 Oxacephems

An effective chemical conversion of the dihydrothiazine ring of **(333)** into the dihydrooxazine ring of oxacephem **(336)** was described by Kim and McGregor[152]. Reaction of cephem **(333; R=H)** with N-chlorosuccinimide in $MeOH/CH_2Cl_2$ gave an 85% yield of the 2-methoxycephem **(333; R=OMe)** which in the presence of Cl_2 in CCl_4 was converted to the aldehyde **(334)**. Alcohol **(335)** was obtained in a 90% yield from reduction of **(334)** with sodium cyanoborohydride in a mixture of THF-acetic acid and ring closure was induced by $AgBF_4$-Ag_2O (1:1) in CH_2Cl_2 furnishing **(336; R=CHPh$_2$)** in 87% yield. Hydrogenation over Pd/C in dioxane/water resulted in the free acid **(336; R=H)** which exhibited significantly decreased biological activity compared to the analogous free acid of **(332)**.



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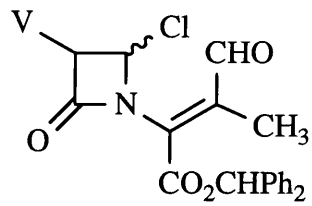

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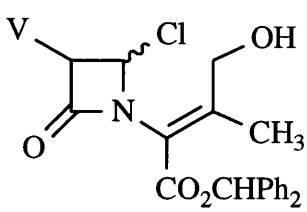

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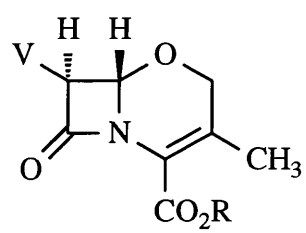
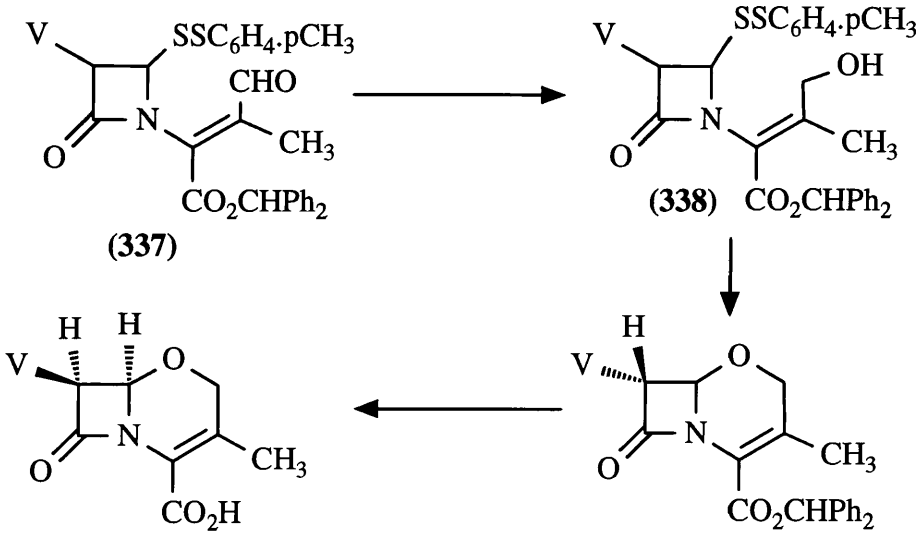
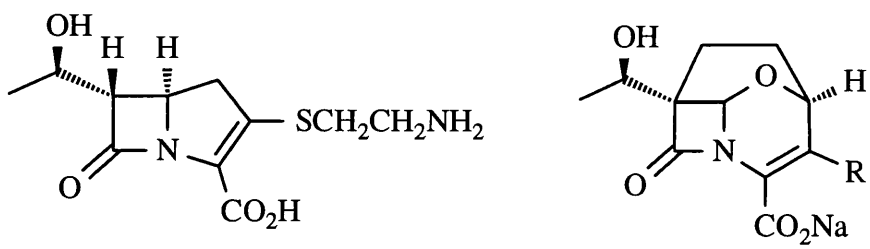


(336)

Another publication describes the conversion of **(337)** into 1-oxacephem acid **(340)** by a similar reduction to the alcohol **(338)** with sodium cyanoborohydride in THF/AcOH[153]. Subsequent ring closure with mercuric trifluoroacetate resulted in the desired 1-oxacephem **(339)** which was epimerised with lithium methoxide and *tert*-butylhypochlorite and de-esterified in the presence of trifluoroacetic acid to afford **(340)**.



Oxacephems that were strictly analogous to thienamycin **(341)** exhibited a weaker potency despite having stronger reactivity and hence Shionogi Labs[154] directed their research towards the three-dimensional structure as another significant influence of biological activity. For this reason they suggested that oxacephems should be of an angled shape such as thienamycin or carbapenem molecules are and thus synthesised tricyclic oxacephem molecules **(342)** bearing the same side-chain as **(341)**. As assumed, the C2-C7 two carbon bridge strongly bent the structure but unfortunately the biological potency was very poor.



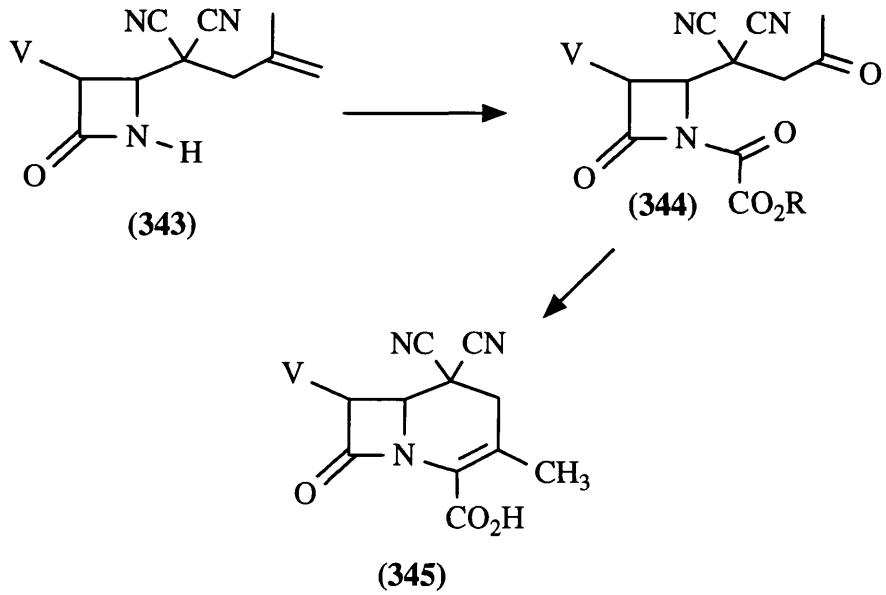
(341) **(342)**

1.7.4.2 Carbacephems

Replacement of the sulphur atom at position 1 of cephalosporins by carbon has been successfully accomplished providing carbacephems which have similar biological activity to their corresponding cephalosporins. However, more

often than not, substitution predominantly results in poorer activity.

According to predictions by Dunlap and colleagues[155], the reactivity and biological activity might possibly be improved by 'increasing the electronegativity of the C-1 carbon'. Thus they describe the synthesis of the 1,1-dicyanocarbacephem (**345**) from (**343**). Ozonolysis of (**343**) at -45°C followed by acylation resulted in (**344**) which when refluxed in xylene in the presence of triethylphosphite and deprotected furnished the desired carbacephem (**345**). As a consequence the dicyanomethylene moiety results in a β-lactam which is much more reactive than corresponding carbacephems or cephalosporins.

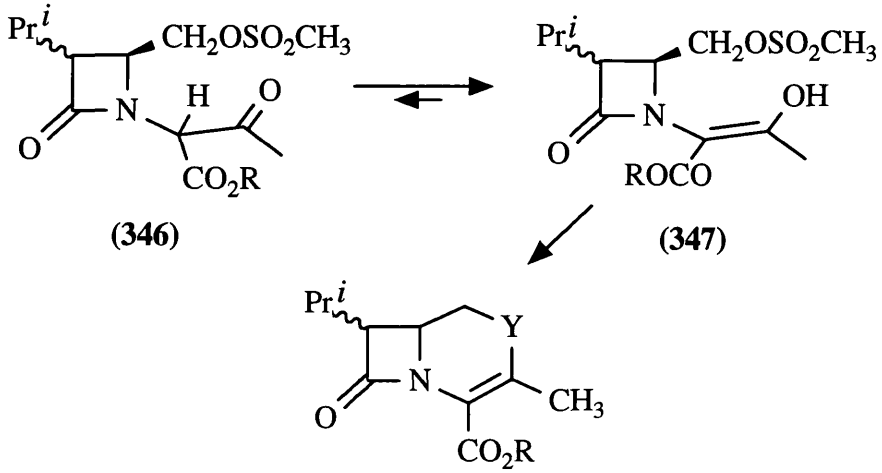


A slight variation on the 1,1-disubstituted carbacephems was the 1,2-disubstituted β-lactams reported by Saito *et al*[156] and yet to be biologically evaluated.

1.7.4.3 Heterocarbacephems

Kajtar-peredy and her Hungarian colleagues[157] cyclised the acetoacetates (**346**) [which exist mainly in their enol forms (**347**)] using triethylamine to afford the 2-oxacarbacephem esters (**348a**). Furthermore methanesulphonation of (**347**) resulted in dimethylsulphonates which were directly transformed into cephem ester (**348b**). Deprotection by the usual procedure

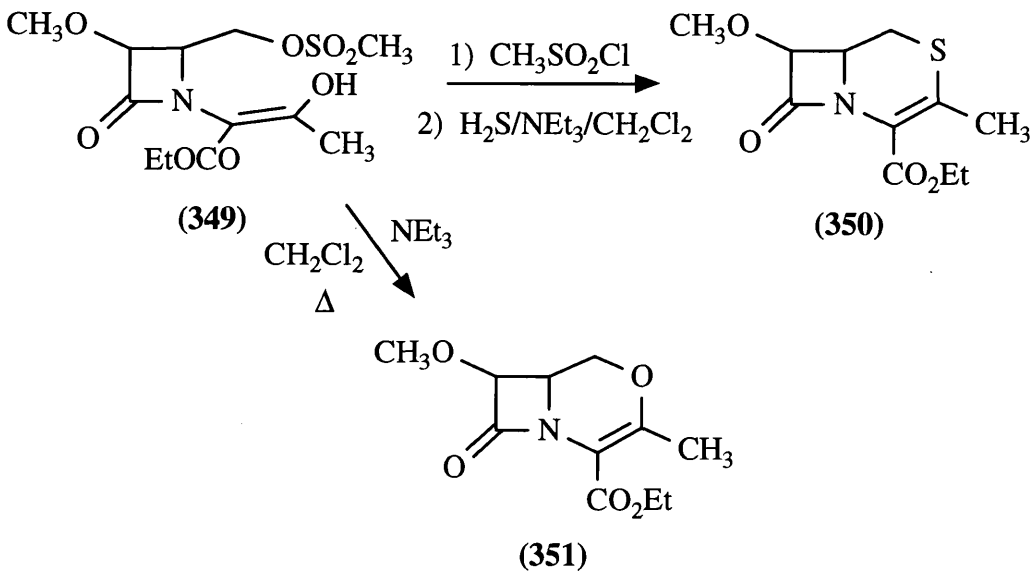
furnished the carboxylic acids which displayed only minimal biological activity.



(346) (347)

(348) a; Y = O
b; Y = S

Applying similar condtions, (**349**) was cyclised to provide 2-thiocarbacephem (**350**) and 2-oxacarbacephem (**351**)[158].

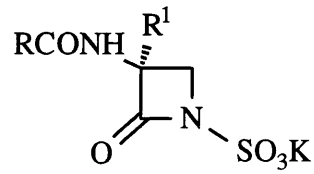


(349) (350)

(351)

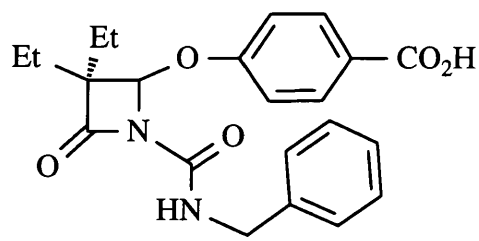
1.7.5 Monobactams

The only known producers of β-lactam antibiotics are fungi and actinomycetes however groups at Takeda[159] and Squibb[160] have, independent of each other, discovered an entirely new class of β-lactam compounds generically termed "monobactams" which are produced by bacteria. They are characterised by the novel 3-acylamino-2-oxoazetidine-1-sulphonic acid eg (**352**). The simplest was



(352)

isolated in 1982[161] as a crystalline potassium salt (**352; R=CH_3, R^1=OCH_3**). In addition monocyclic β-lactams eg (**13 &** derivatives **353**) have become the first orally potent inhibitors of HLE - an enzyme that causes the degradation of lung tissue[162].



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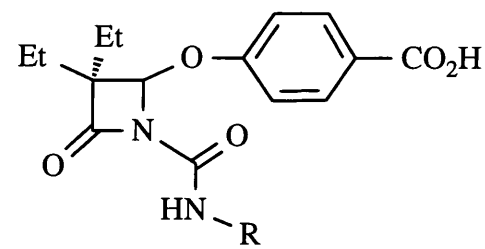


(353)

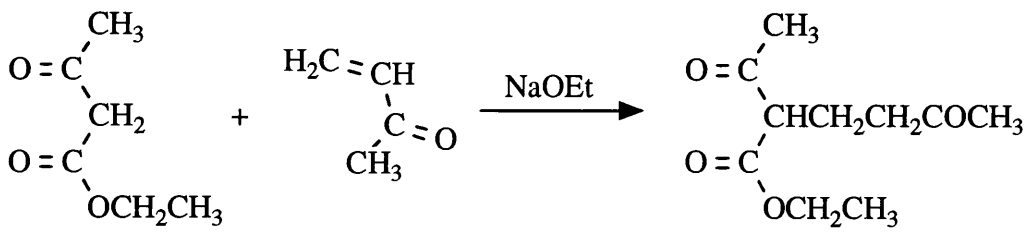
DISCUSSION

2.0 DISCUSSION

The main aim of this project was to emulate clavulanic acid (see Section 2.3) by designing ceph-3-ems which incorporate a C2-exocyclic double bond and a good leaving group.

2.1 Reactions with Michael Acceptors

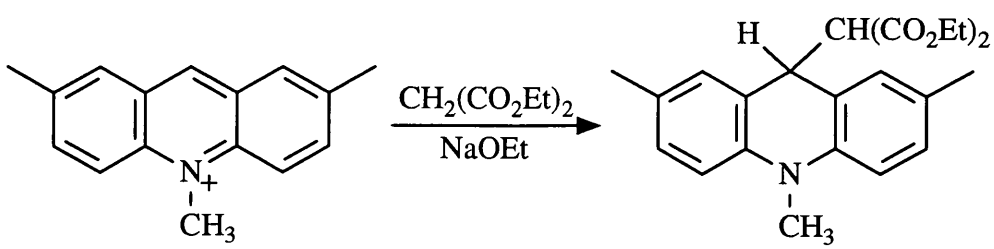
Reaction of an enolate ion nucleophile with α,β-unsaturated carbonyl electrophiles is known as the Michael reaction[163]. The best and most useful condensations involve stabilised enolate ions derived from dialkyl malonates which add to unhindered α,β-unsaturated ketones eg the addition of ethyl acetoacetate to 3-buten-2-one in the presence of sodium ethoxide resulting in **(354)** in a 94% yield as shown in Scheme 1.



Scheme 1 **(354)**

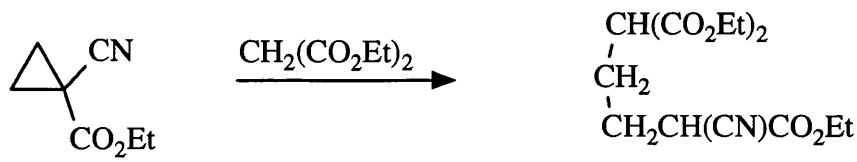
Originally the Michael condensation was considered as the addition of an activated methyl group to α,β-unsaturated aldehydes, ketones, esters or acid derivatives. An extension of this original thinking includes groups such as nitriles, sulphones or as shown below quarternary ammonium salts.

The unsaturated cyclic quarternary ammonium salt **(355)** reacts with diethyl malonate in the presence of sodium ethoxide to afford **(356)**[165].



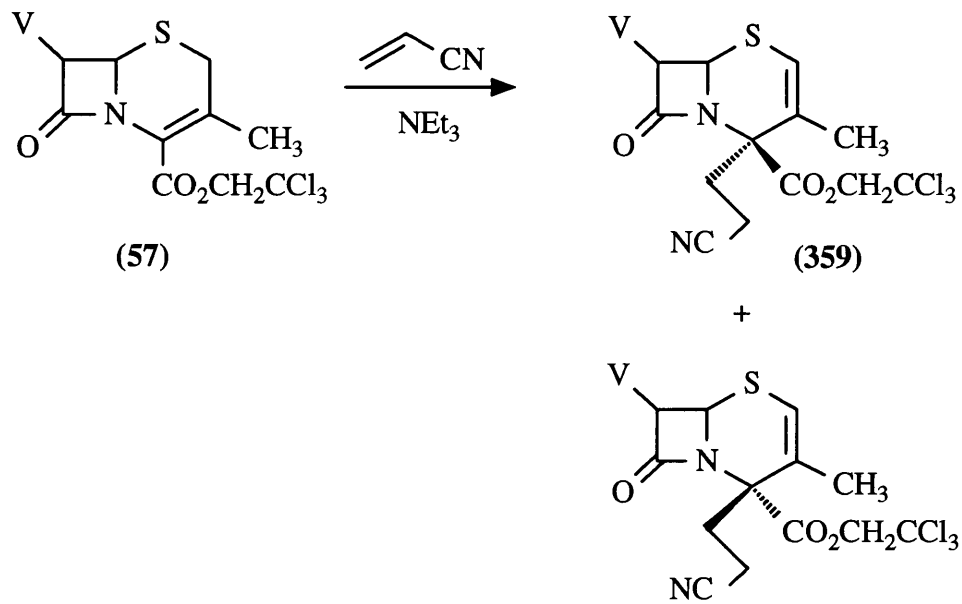
(355) **(356)**

Some cyclopropane derivatives have participated in the Michael reaction[166] eg ethyl 1-cyanocyclopropane 1-carboxylate (**357**) undergoes condensation with diethyl malonate to produce (**358**).



(**357**) (**358**)

Also, the system C=C-C=N behaves like the system C=C-C=O and can undergo Michael condensations with enolate ion nucleophiles. This has been demonstrated[59] in the addition of acrylonitrile with ceph-3-ems, and predominantly involved addition at the C-4 position. Thus, treatment of the trichloroethyl ester (**57**) with a catalytic amount of triethylamine in acrylonitrile resulted in the C-4 substituted α- and β-isomers (**359**) and (**360**)[60]. As a result of in-depth studies by



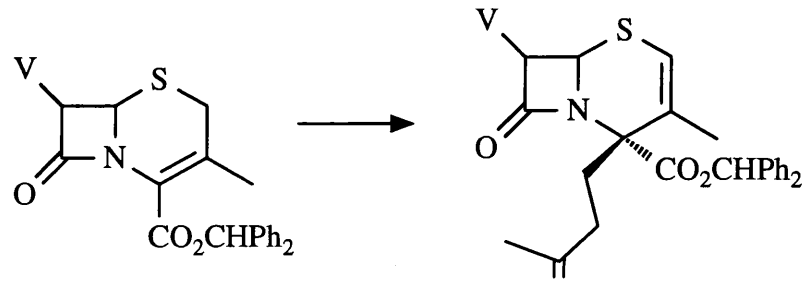
(**57**) (**359**)

+

(**360**)

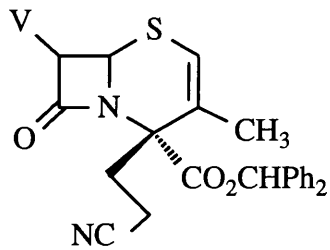
Yoshida *et al*[85] into C-4 methylthiolation reactions, β-stereochemistry was assigned to the major product. Reaction of the diphenylmethyl ester (**361**) with methyl vinyl ketone in the presence of triethylamine gave only one of the two

possible isomers, namely the 4β-adduct (**44**) in a high yield of 90%.

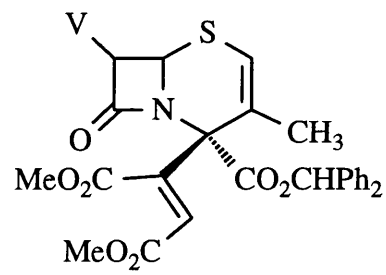


(**361**) (**44**)

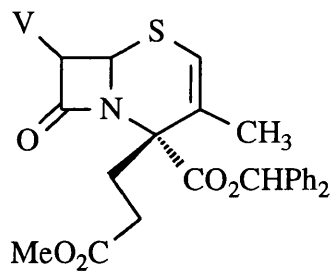
Subsequent to this report[88], the Michael adduct (**362**) was obtained in a 74% yield from addition of acrylonitrile with the ceph-3-em (**361**) in the presence of triethylamine. Similarly, reaction with dimethyl butynedioate gave (**363**) in a reasonable yield of 40% and methyl acrylate afforded ceph-3-em (**364**) in 53% yield but only when Triton B was employed as base.



(**362**)

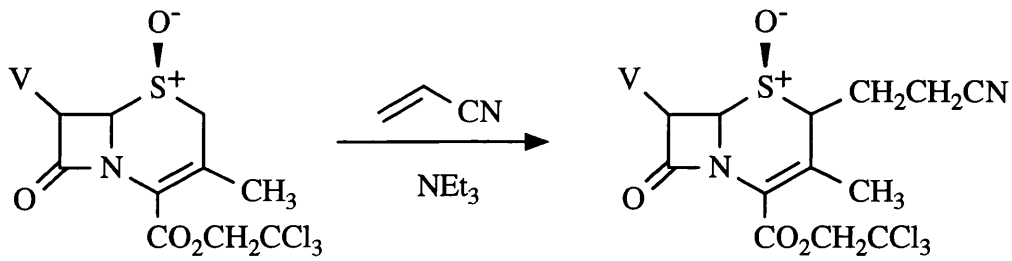


(**363**)

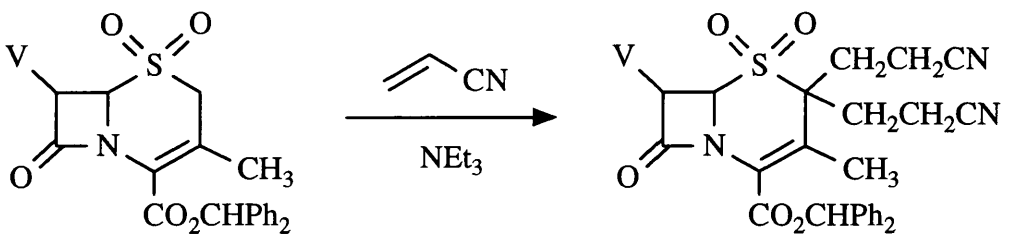


(**364**)

Furthermore, acrylonitrile has undergone Michael additions with the S-sulphoxide of the trichloroethyl ester and with the sulphone of the diphenylmethyl ester. Reaction of the sulphoxide (**51**) proceeded slowly (only in the presence of triethylamine) and resulted in a poor yield (24%) of adduct (**365**) whereas, reaction using the sulphone (**366**) afforded the diadduct (**367**) in 54% yield.



(51) (365)

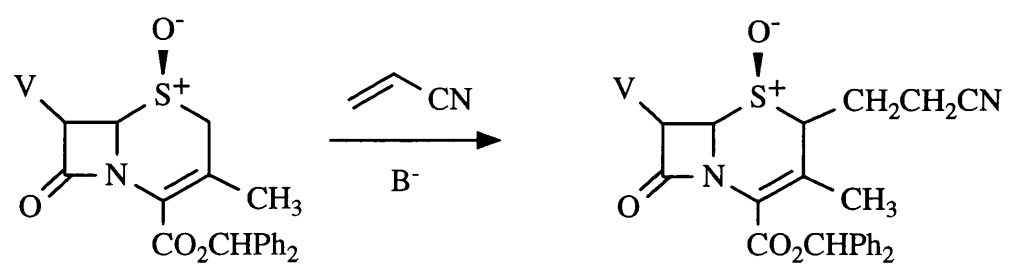


(366) (367)

2.1.1 Michael Reactions of Ceph-3-em Sulphoxides

Acrylonitrile

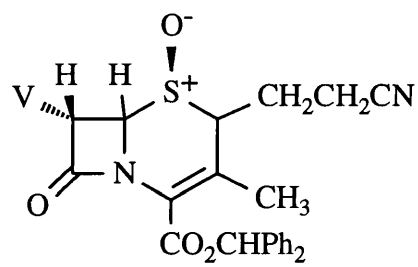
As a result of the extensive research carried out on the Michael addition of acrylonitrile with the trichloroethyl ester it was decided to extend this work to the sulphoxide of the diphenylmethyl ester **(202)** in an attempt to prepare the C-2 Michael adduct **(368)** and, hence, try to form 2-exomethylene derivatives (Section 2.3). Thus, in this project, sulphoxide **(202)** was stirred overnight in acrylonitrile



(202) **(368)**

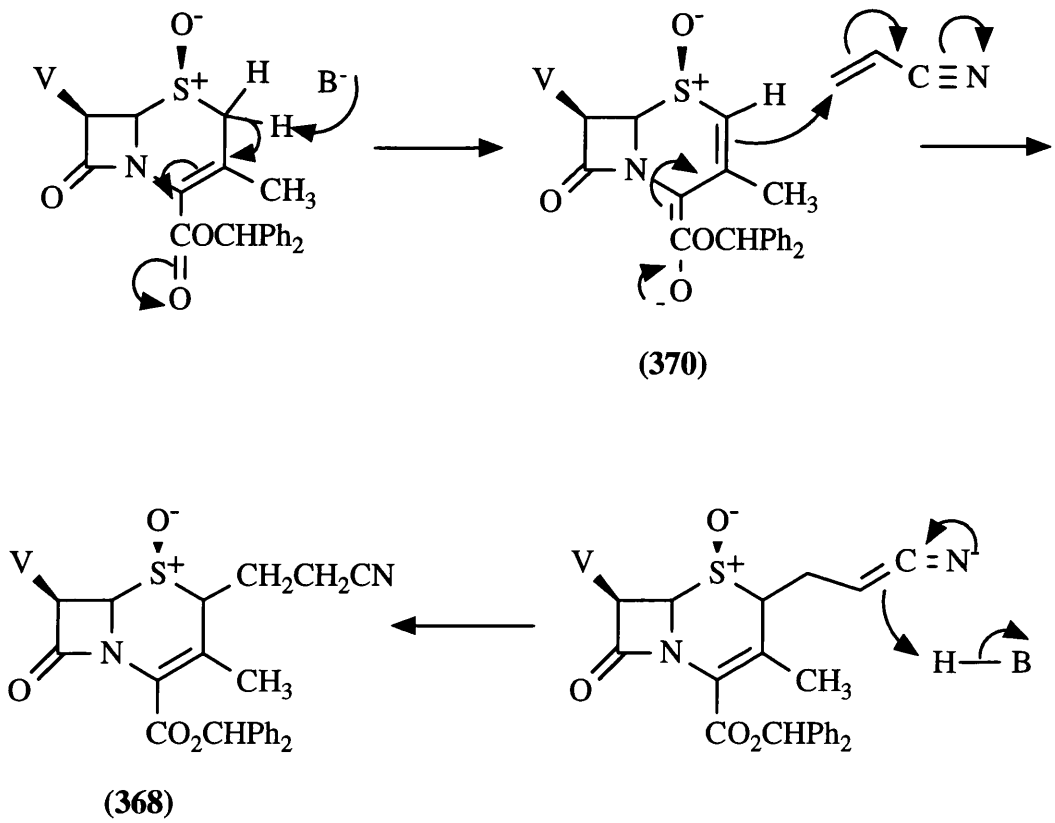
with triethylamine but (according to tlc) the majority of the starting material remained unreacted, although there were a number of other compounds present. Short path chromatography was attempted but no homogenous products could be

isolated probably due to the complexity of the mixture. In order to try and prevent numerous compounds being produced, the reaction time was reduced and gentle heating was introduced to encourage addition. Again tlc indicated a complex mixture of products, however, a minor component which contained the β-lactam ring according to ir (1791 cm^{-1}) was obtained after column chromatography. A sharp peak at 2248cm^{-1} on the infrared spectrum indicated the presence of the nitrile group and nmr displayed a multiplet integrating for 2 protons at δ1.19-1.59 and a multiplet at δ2.43-2.52 which also integrated for 2 protons, denoting the incorporation of a single molecule of acrylonitrile. Furthermore, a singlet was observed at δ1.91 for 3-CH_3 indicating the double bond had remained in the 3,4-position and addition had occurred at the desired C-2. Unfortunately, a noticeable reduction in the coupling frequency for protons at the C-6 and C-7 positions was also observed indicting that epimerisation at C-7 has occurred. Hence the product isolated from this reaction was assigned the structure **(369)** on the basis of nmr information. Mass spectroscopy gave a molecular ion of 606 (MNa^+) which is consistent with structure **(369)** displaying a molecular weight of 583.



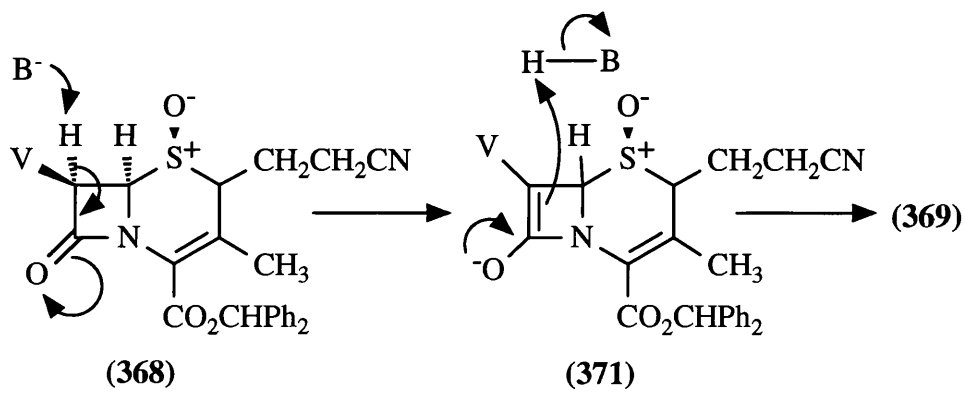
(369)

The reaction mechanism possibly involves the removal of a C-2 hydrogen by triethylamine and delocalisation of electrons into the ester group creating the nucleophile **(370)** which attacks the Michael acceptor displacing electrons into the nitrile group. Protonation affords the desired Michael adduct **(368)** as shown in Scheme 2.



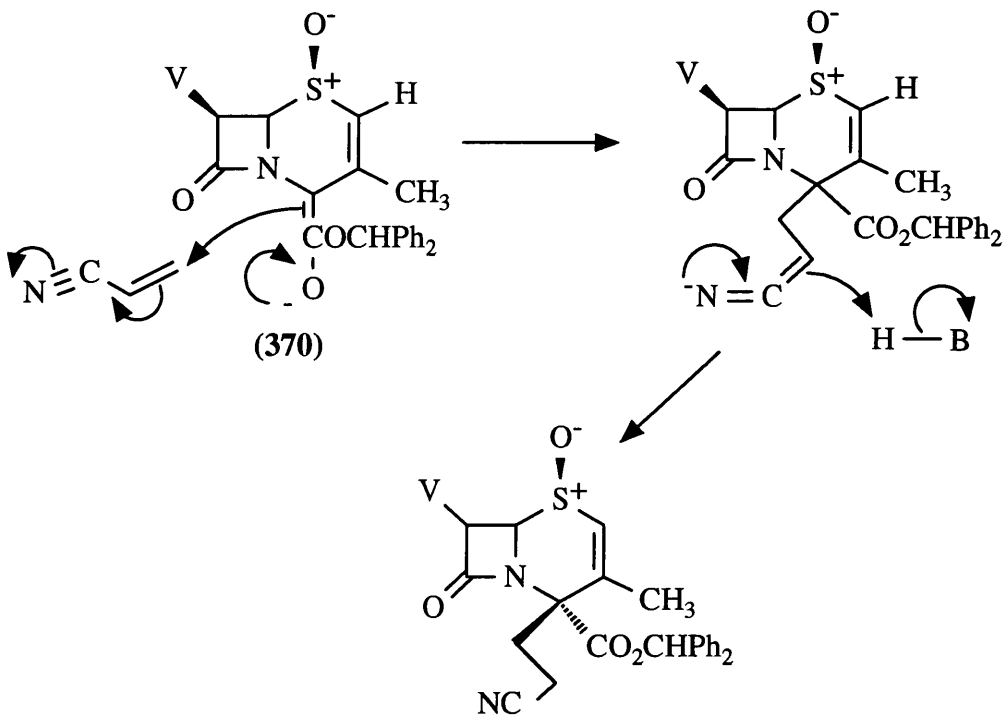
Scheme 2

Similiarly the base removes an acidic hydrogen from position 7 and the electrons are delocalised into the β-lactam carbonyl creating a planar molecule. In this case the generated nucleophile **(371)** protonates from the less hindered β-face to give **(369)** as shown in Scheme 3.



Scheme 3

Numerous reactions can occur within this system and it is understandable why it culminated in a complex mixture of products. There exists the possibility that nucleophile **(371)** may also undergo a Michael reaction leading to a 7-substituted adduct or a 2,7-disubstituted adduct (if addition has already taken place at the 2-carbon). It is also apparent from Scheme 2 that substitution can also occur at the C-4 position. Nucleophile **(370)** can attack from C-4 resulting in the 4β-(2'-cyanoethyl) ceph-2-em **(372)** (Scheme 4).



(370)

(372)

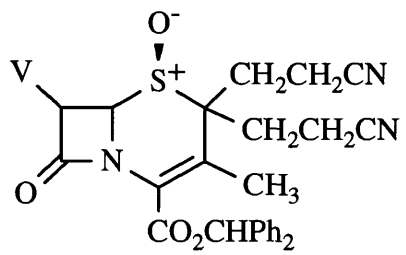
Scheme 4

Further complications arise from the presence of a second C-2 hydrogen which can also be removed and, hence, lead to either 2,2- or 2,4-disubstituted products.

To establish the best time to terminate the reaction ie when the reaction mixture was still clean enough to allow successful chromatography, an experiment was performed as before and analysed by tlc every 10 minutes for the first hour; every 15 minutes for the following two hours; and every 30 minutes for the last

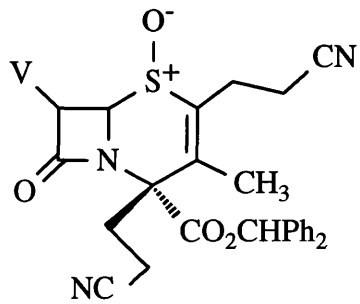
two hours. Within the first hour very little reaction occurred. The majority of starting material remained unreacted and a slight build up of 2 less polar products was observed. Over the next two hours, the appearance of 4 more products was observed in addition to baseline material and during the last two hours the reaction mixture became streaky on tlc. Thus the critical part of the reaction was in the first two hours. Therefore to encourage reaction to take place within this time the sulphoxide **(202)** was heated at reflux with acrylonitrile in the presence of triethylamine for 2 hours, however tlc revealed the existence of numerous components inseparable by column chromatography.

In contrast to reports by Bremner *et al*[60], who discovered triethylamine was a more favourable base when reacting the trichloroethyl ester **(51)** with acrylonitrile, triethylamine is not an effective base in the case of the diphenylmethyl ester. As a result, Triton B was employed and added to the sulphoxide **(202)** suspended in acrylonitrile. After 1 hour, two less polar spots appeared (in larger quantities than with triethylamine) and rather than allow the reaction to degrade, it was worked up. Two products were isolated via chromatography of which the least polar was a white crystalline solid that displayed the β-lactam carbonyl stretching frequency at 1781cm^{-1}. From nmr spectroscopy, a multiplet was observed over the range δ2.8-3.01 which integrated for 8 protons indicating the incorporation of two acrylonitrile residues. The remainder of the spectrum was in accord with the structure **(373)** and supported by mass spectroscopy displaying the molecular ion 659.5 for MNa^+.



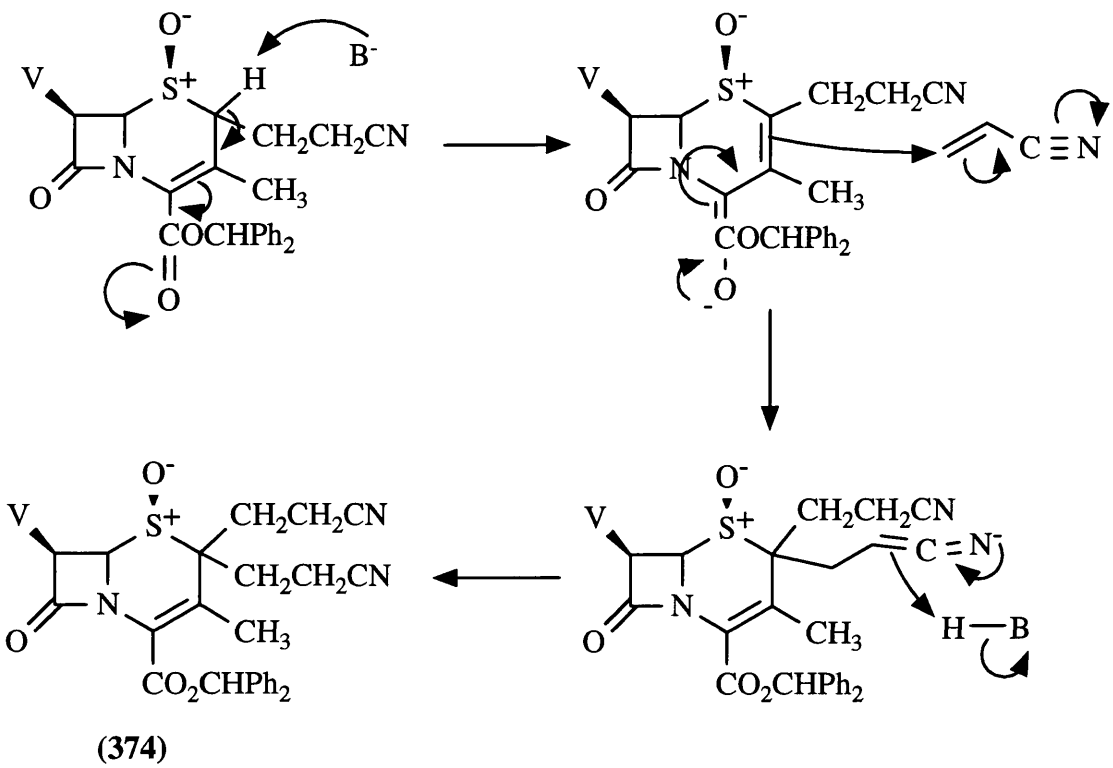
(373)

There also exists the possibility of the second acrylonitrile molecule being incorporated in the C-4 position resulting in the 2,4-diadduct (**374**). However on the basis of previous literature[60] the 2,2-disubstituted structure is assigned.



(374)

The first addition occurs as in Scheme 2 and the second by the removal of the remaining C-2 hydrogen as shown in Scheme 5.



(374)

Scheme 5

The second component from this reaction gave the same molecular ion as (**369**) and the nmr spectrum, although very similiar to (**369**), contained one

distinct difference ie the coupling frequency for the C-6 and C-7 protons was 4.8 Hz indicating no epimerisation had occurred at C-7 leaving the only feasible stucture as **(368)** produced (in 17% yield) according to Scheme 2. Further confirmation for this stucture was given by microanalysis in which the observed values for C, H, N and S were virtually identical to the required figures for $C_{32}H_{29}N_3O_6S$.

A similiar reaction was carried out with sulphoxide **(202)** and acrylonitrile, again using the base - Triton B, and the reaction time was increased to 24 hours. The 2,2-diadduct **(373)** was obtained in 70% yield and the 2-substituted ceph-3-em **(368)** in 6%. Additionally, a mixture of two less polar components was collected from the reaction. However they were inseparable from each other due to the small difference in their Rf values. It was speculated that oxidising the mixture to their respective sulphones might increase this difference and enable successful separation by chromatography. Therefore, the crude reaction mixture was reacted with *m*-CPBA in dichloromethane. Two less polar spots were observed on tlc but the small difference between their Rf values remained and column chromatography was not attempted. No further studies were carried out to elucidate their structures.

2-Chloroacrylonitrile

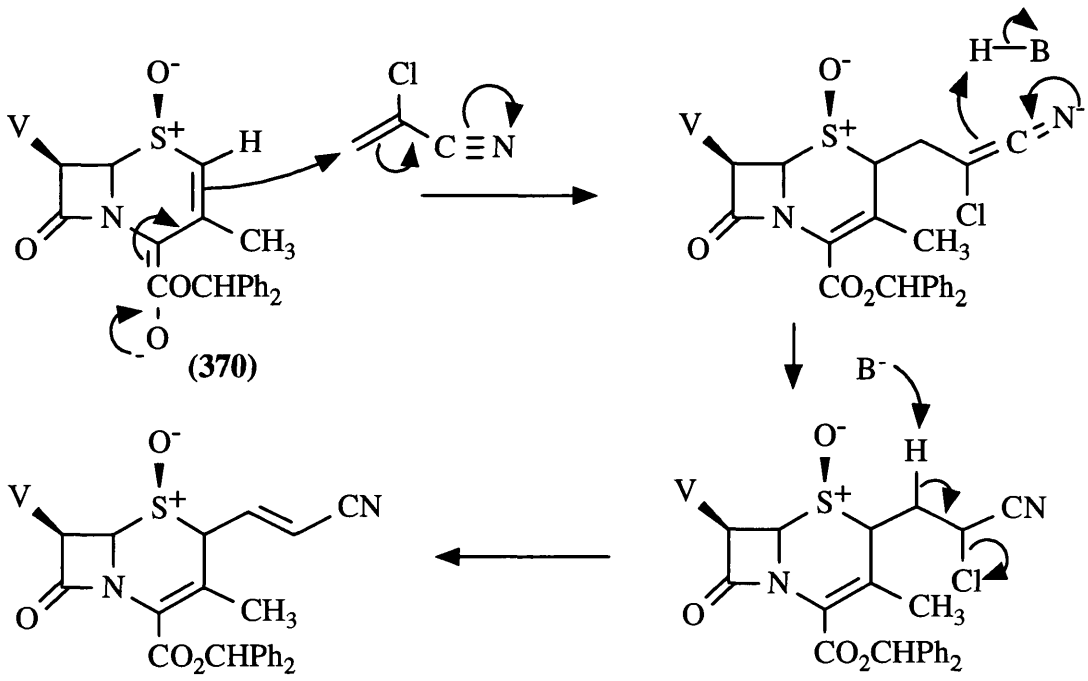
It was considered that 2-chloroacrylonitrile would be more susceptible to nucleophilic attack than acrylonitrile and therefore result in a cleaner reaction mixture with the possibility of an easy separation of products and so reaction of **(202)** was considered with this Michael acceptor.

Initial reactions in dry THF with triethylamine were unsuccessful and the sulphoxide **(202)** was recovered unreacted. Using sodium hydride and DMF, tlc indicated the presence of numerous products which were inseparable by column chromatography. As in the Michael addition of acrylonitrile, 2-chloroacrylonitrile

was employed as the solvent. Thus, ceph-3-em (**202**) was directly suspended in 2-chloroacrylonitrile with triethylamine as base. Over a period of 5 hours the reaction solution darkened considerably and ir spectroscopy showed complete lack of the β-lactam carbonyl signal.

Modifying the conditions to stirring the sulphoxide, 2-chloroacrylonitrile and triethylamine in acetonitrile overnight at room temperature, afforded four β-lactam containing products. All displayed the presence of a nitrile group (according to ir) and gave similiar nmr spectra ie multiplets from δ1.8-2.3 integrating for 3 protons indicating all four products had incorporated a 3 proton substituent containing a nitrile group. Signals for the side chain, ester group and 3-CH_3 were as required for a ceph-3-em nucleus. The differences arose in nmr signals for H-6 and H-7. Two compounds displayed the usual doublet and double doublet with coupling constants of 4.7 Hz which is normal for 6R, 7R stereochemistry, however the remaining two products showed coupling constants of 2.3 Hz indicating epimerisation had occurred and 6R, 7S stereochemistry has resulted. Furthermore no chloride atom was present according to a sodium fusion test carried out on the four products. From mass spectroscopy all four revealed molecular ions of 599 for MNH_4^+ indicating that the molecular weight would be 581 amu implying the molecular formula for the four isomers as $C_{32}H_{27}N_3O_6S$.

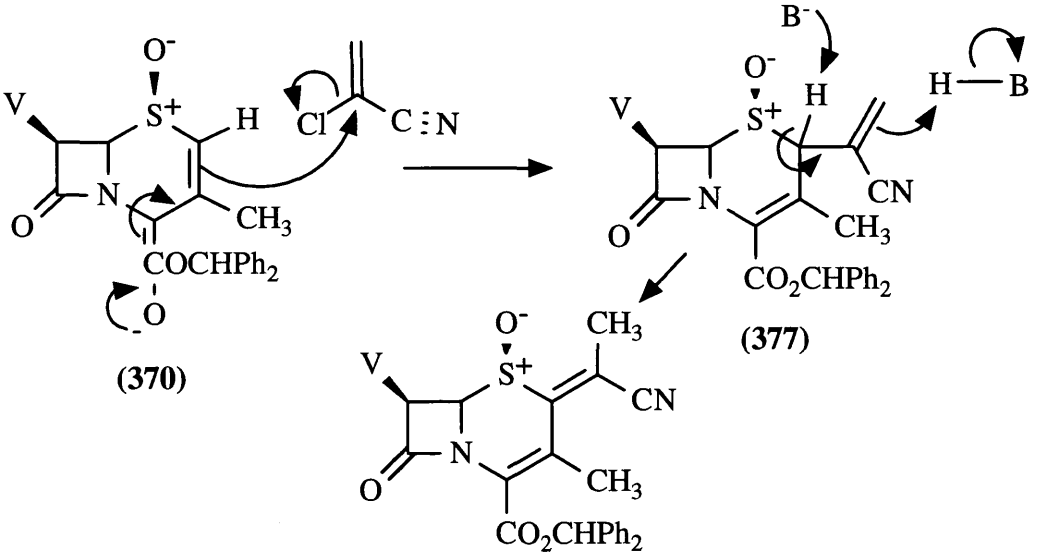
The expected reaction mechanism is shown in Scheme 6 ie removal of the C-2 hydrogen creating the C-2 nucleophile (**370**) which attacks the 2-chloroacrylonitrile and gives product (**375**) containing 4 extra protons. Elimination of the chloride ion affords (**376**) with the required 3 protons at C-2. However, from the nmr spectra, the chemical shifts for these 3 protons suggests a saturated system.



(370) (376) (375)

Scheme 6

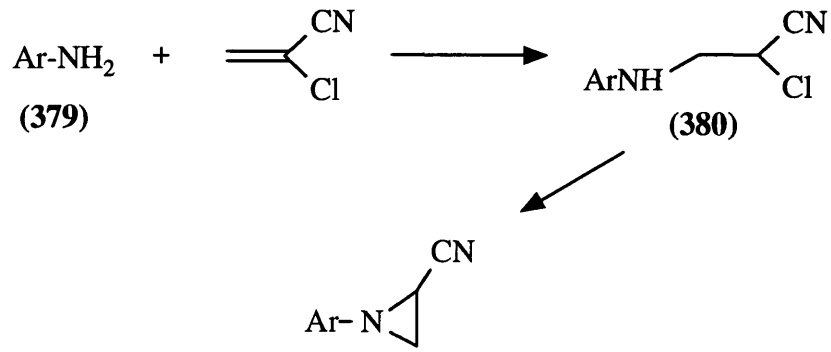
Another possibility is nucleophilic attack of the second carbon displacing the chloride ion (Scheme 7) but again this leads to an unsaturated system **(377)**. Isomerisation of **(377)** to **(378)** would result in a more stable compound with conjugated double bonds but nmr would display the methyl group as a singlet.



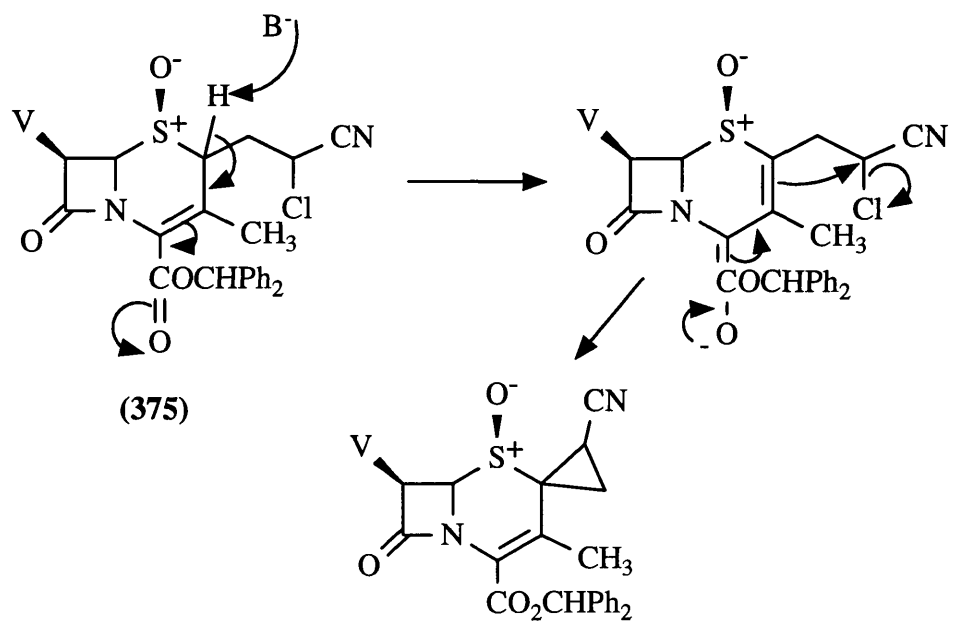
(370) **(377)** **(378)**

Scheme 7

An examination of the relevant literature[166] revealed a reaction of an aryl amine **(379)** with 2-chloroacrylonitrile. The Michael adduct **(380)** was obtained which cyclised to the cyclopropyl derivative **(381)**. Thus, Michael adduct



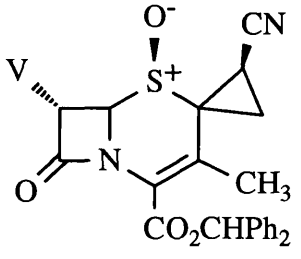
(375) contains another C-2 proton which on removal by the excess triethylamine present results in another resonance stabilised C-2 anion. Nucleophilic attack, followed by elimination of the chloride ion, would culminate in the 2-cyclopropyl structure **(382)** as shown in Scheme 8.



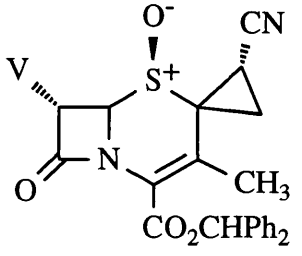
Scheme 8

Structure **(382)** incorporates 3 protons at C-2 in a saturated system and satisfies the required molecular formula. Furthermore compound **(382)** contains a new chiral centre and consequently, the 4 products from Michael addition of sulphoxide **(202)** with 2-chloroacrylonitrile, are thought to be diastereoisomers and epimers of structure **(382)**.

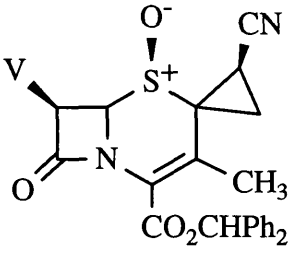
It is speculated that the two major products are the C-7 epimers **(382a)** and **(382b)** afforded in 25% and 18% yields and their corresponding isomers **(382c)** and **(382d)** were obtained in 13% and 7% yields respectively.



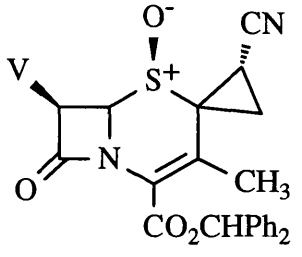
(382a)



(382b)



(382c)

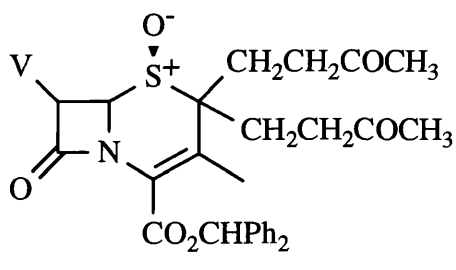


(382d)

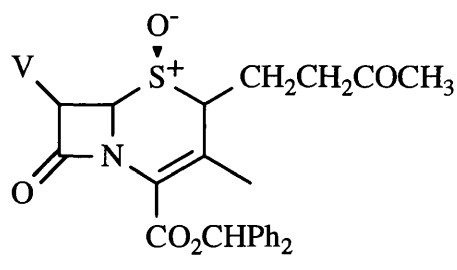
Methyl Vinyl Ketone

Methyl vinyl ketone is an important Michael acceptor which has been reviewed[167] extensively and previously reacted successfully at the C-4 position of the ceph-3-em sulphide. As a consequence, Michael addition of methyl vinyl ketone to the sulphoxide **(202)** was attempted. However, unlike addition to the sulphide, the reaction occurred slowly and only after a large excess of triethylamine was added. Two products were isolated by chromatography and the

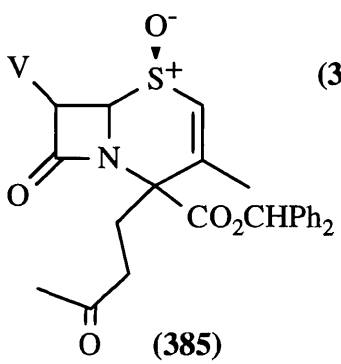
nmr for the major component displayed the incorporation of two methyl vinyl ketone molecules. Two singlet peaks (integrating for 3 protons each) were observed at δ2.12 & δ2.14 and assigned to the methyl groups from the methyl vinyl ketone and a multiplet at δ2.5-3.1 for 8 protons was evidence of CH_2CH_2CO functionality. From nmr spectroscopy, which was supported by microanalysis and mass spectroscopy (both indicating the molecular formula to be $C_{37}H_{38}N_2O_8S$), the major product was identified as the 2,2-disubstituted compound **(383)**. This confirmed the report[168] that one of the problems of methyl vinyl ketone is its willingness to di-alkylate active carbons. Another restriction[168] of this Michael acceptor is that alkylation usually takes place at the more highly substituted carbon - a condition displayed by the second isolated product which was expected to be the 2-alkylated ceph-3-em **(384)**. The nmr spectrum showed one singlet at δ2.15 integrating for 3 protons and a multiplet at δ2.5-3.1 for 4 protons indicating the presence of only one mole of methyl vinyl ketone. Additionally vinylic coupling between the C-3 methyl group and the single C-2 proton was observed (0.9 Hz) confirming the unexpected ceph-2-em structure and hence the 4-substituted adduct **(385)**. Mass spectroscopy and elemental analysis data corroborated this structure.



(383)



(384)

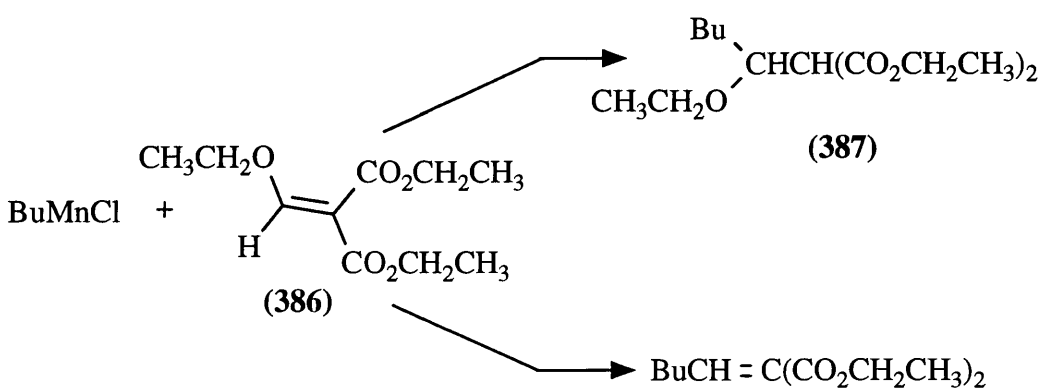


(385)

It is worth noting that for a di-adduct to be produced, alkylation must first have occurred at C-2 to give a ceph-3-em structure which is able to undergo a second addition. Therefore, at some point during this reaction the 2-adduct **(384)** was present and because of the large quantities of base, all possible **(384)** was converted to **(383)**. However, when base was minimised, again the di-adduct **(383)** and the mono-adduct **(385)** were the only products from this reaction.

Diethyl Ethoxymethylenemalonate

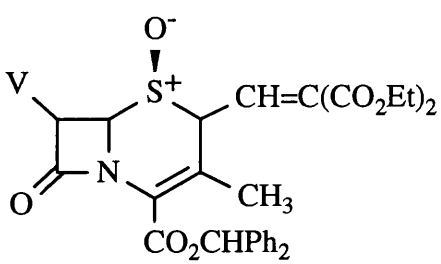
Diethyl ethoxymethylenemalonate **(386)** is another good Michael acceptor which, having its double bond conjugated to two ester moieties, makes it more susceptible to nucleophilic attack at the β-carbon. In addition the intermediate formed is further stabilised by the extra ester group. A report[169] on this type of reaction with organomanganese reagents verified the reactivity of this diester. Thus, addition of butylmanganese chloride to **(386)** in THF at 0°C over a period of 10 mins followed by neutral hydrolysis resulted in the product **(387)** in a high yield of 86%. Under the same initial conditions followed by acid hydrolysis using 1M HCl, the addition-elimination product **(388)** was afforded, again in a high yield of 87%.



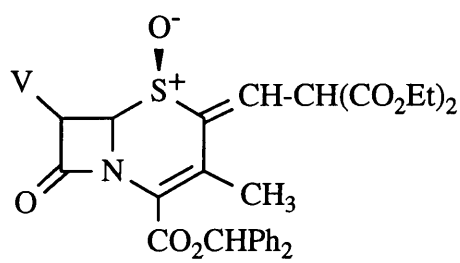
When diethyl ethoxymethylenemalonate was added to a solution of the ceph-3-em sulphoxide **(202)** in dry THF using triethylamine as the base no reaction

was observed according to tlc. A similiar reaction was attempted in the presence of the stronger base Triton B but degradation of the β-lactam ring occurred. It was decided as in previous reactions to increase the concentration of the Michael reagent by suspending the sulphoxide (**202**) directly in diethyl ethoxymethylenemalonate with triethylamine present as base. Reaction occurred slowly over a period of 15 hour and tlc showed the presence of one major and two minor products as well as unreacted starting material, which were isolated after rapid short-path chromatography.

The nmr spectrum for the least polar product showed the presence of two ethyl groups corresponding to the incorporation of a diethyl malonate residue. It also indicated two downfield hydrogens as doublets coupling with each other as part of an unsaturated system. This information suggested two possible structures (**389**) and (**390**). However, the oil obtained was highly yellow in colour and as



(**389**)

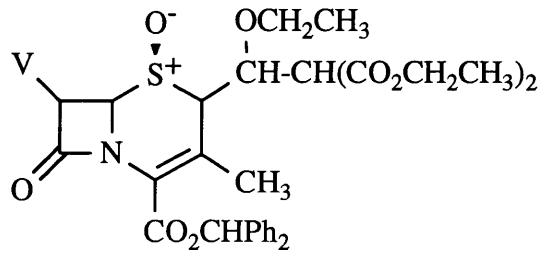


(**390**)

previous C-2 exomethylene ceph-3-ems were also highly coloured[181] (as a consequence of conjugation with the Δ3-double bond), structure (**390**) was assigned. Further evidence to corroborate this structure was obtained from the mass spectrum which displayed a fragment of mass 86 corresponding to $CHCO_2CH_2CH_3$ - a fragment that does not exist in structure (**389**). Elemental analysis was also consistent with structure (**390**).

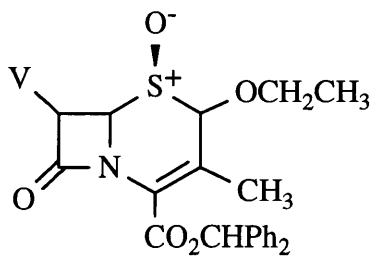
The next product eluted had a similiar nmr spectrum to (**390**) ie it comprised of a multiplet at δ1.3 integrating for 9 protons and a multiplet at δ4.25 integrating for 4 protons. In addition, a multiplet at δ3.4, integrating for 2 protons,

indicates the presence of both ester groups and the ethoxy group. Therefore structure **(391)** is assigned and this is supported by mass spectroscopy. Microanalytical figures were not in agreement with this structure. However, the presence of residual solvents in product **(391)** were obvious from the nmr spectrum and attempts at crystallisation were unsuccessful.



(391)

The final product isolated from this reaction was a white crystalline solid (mp 203-205°C) that incorporated only one ethyl group according to nmr and the downfield position of the CH_2 protons suggests the ethyl group is adjacent to an electronegative atom as opposed to an alkyl group and therefore structure **(392)** is proposed. Microanalysis figures for C, H and N were consistent with this

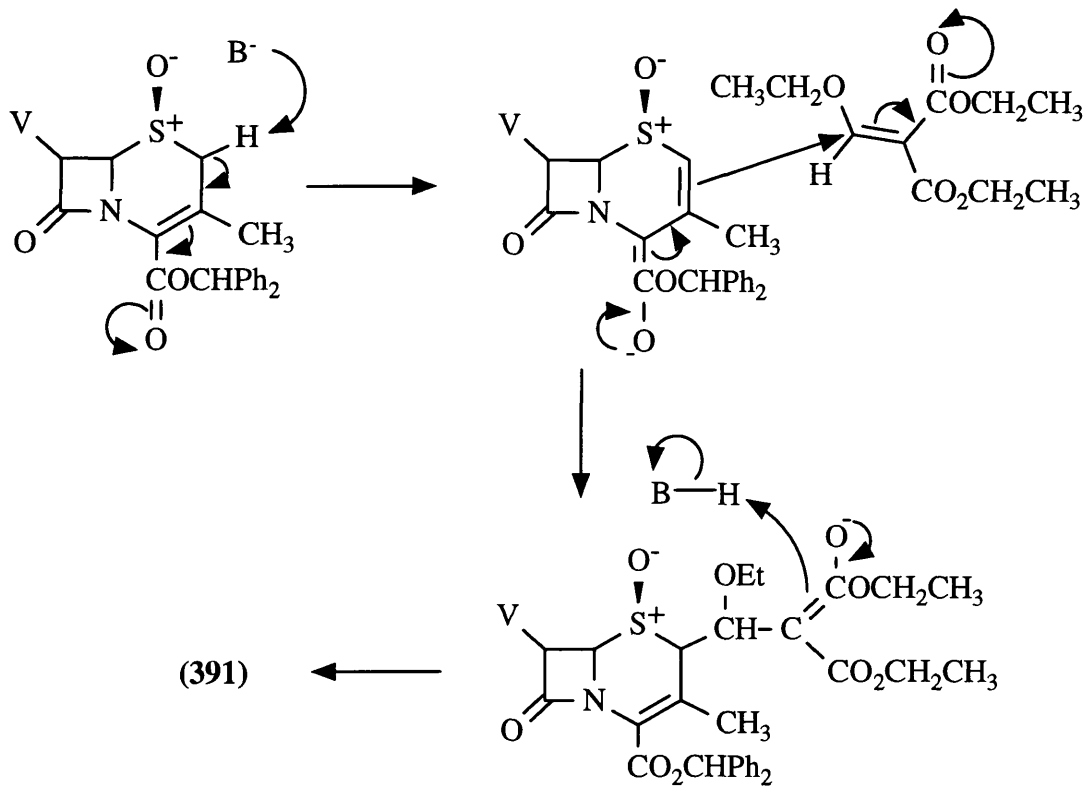


(392)

structure and although no molecular ion was present in the mass spectrum, a fragment of mass 408 which is equivalent to the molecular ion minus the diphenylmethyl ester group was observed.

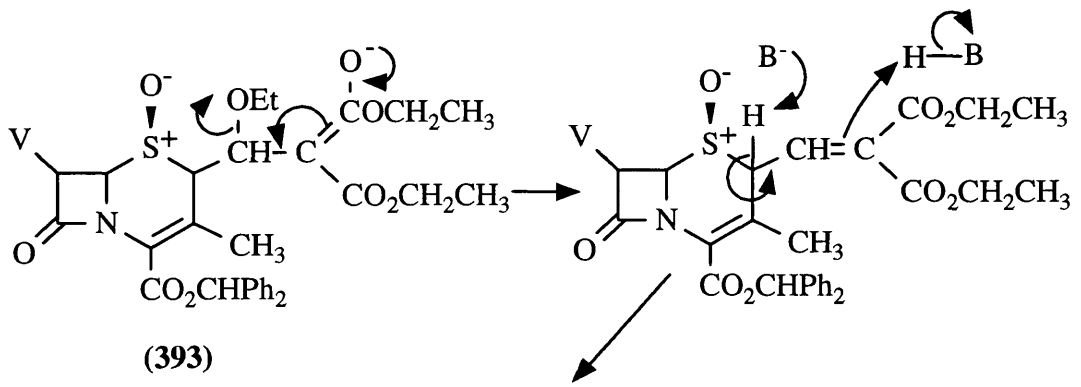
Scheme 9 shows a possible mechanism for the synthesis of **(391)**. The C-2 hydrogen being the most acidic is removed by triethylamine, and the electrons are displaced into the carbonyl group of the ester creating a nucleophilic site at

C-2. Attack at the carbon-carbon double bond of diethyl ethoxymethylenemalonate delocalises the electrons into the ester groups producing intermediate **(393)** which protonates to give **(391)**.



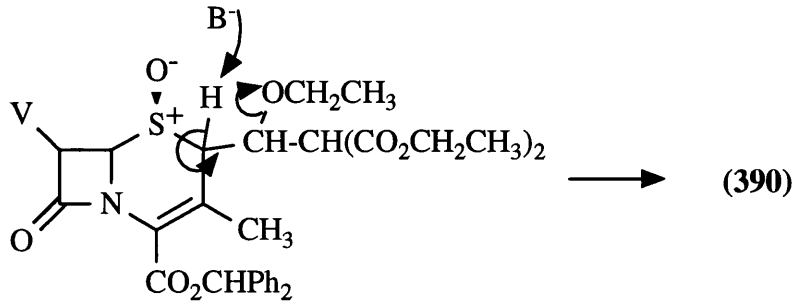
Scheme 9

Three possible routes exist for the formation of **(390)**. First, direct elimination of the ethoxy group from intermediate **(393)** followed by rearrangement to give the desired product (Scheme 10).



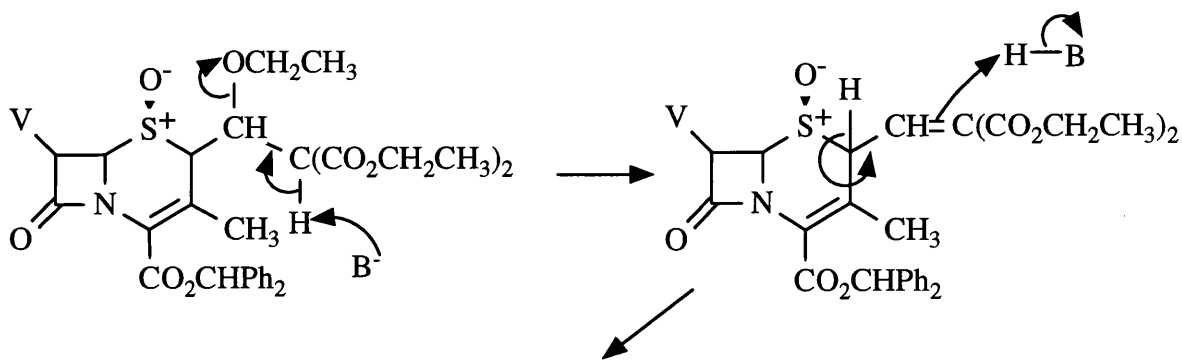
Scheme 10

Alternatively, triethylamine could remove either the C-2 hydrogen from **(391)** inducing ethoxy elimination (Scheme 11) or the hydrogen between the two



Scheme 11

ethyl esters (Scheme 12) again resulting in the elimination of the ethoxy group which, followed by rearrangement, gives the desired C-2 exomethylene compound **(390)**.



Scheme 12

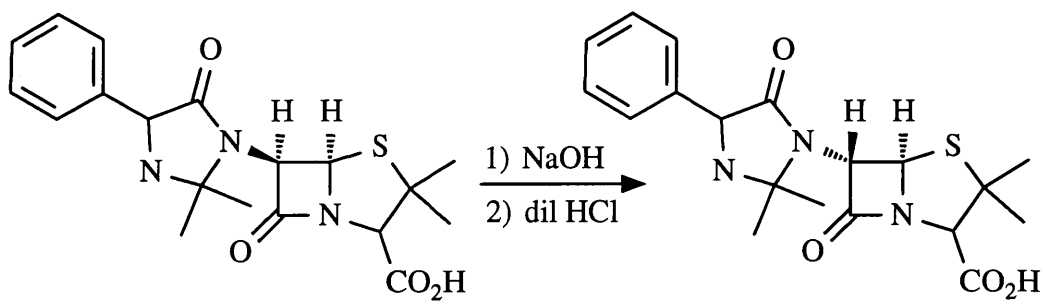
In an attempt to confirm the latter mechanism, the sulphoxide **(391)** was dissolved in dry THF and triethylamine added. However no reaction was observed at room temperature (tlc) and heating the reaction mixture at reflux resulted in a complex mixture of products. This experiment thus provides evidence for the mechanism shown in Scheme 10.

A major problem encountered with Michael addition using the diethyl ethoxymethylenemalonate reagent was separating the products from the malonate which had a boiling point of 280°C. Thus, an attempt at simplifying the separation of the Michael adduct from the reaction products using bulb to bulb distillation

was undertaken. Unfortunately, Michael adducts **(390)** and **(391)** rapidly degraded, though product **(392)** was isolated cleanly in a 22% yield. Another attempt involved chromatographing the crude reaction mixture slowly over a period of 4 days. A successful separation of the Michael acceptor from the reaction mixture was accomplished but only product **(390)** was obtained.

2.1.2 Attempted Epimerisation at C-7

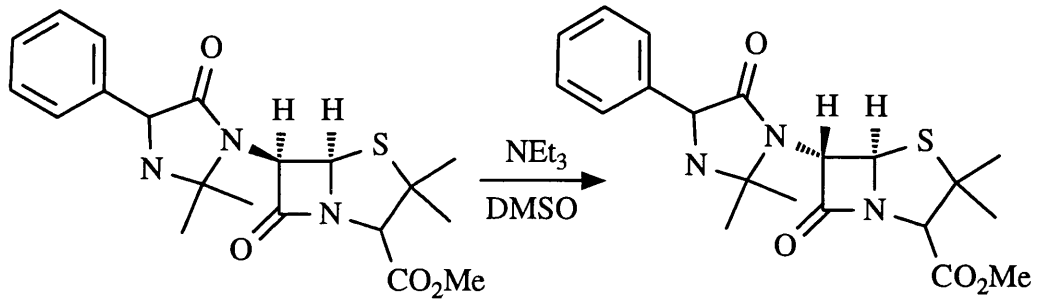
Epimerisation of the C-7 proton (as was observed in the Michael addition of 2-chloroacrylonitrile with ester **(202)**) was first reported in 1968[170] when the antibiotic hetacillin **(394)** on treatment with aqueous sodium hydroxide for 30 minutes, followed by dilute hydrochloric acid, afforded epihetacillin **(395)** in 85% yield. It was noted that both compounds exhibited similiar nmr spectra



(394) **(395)**

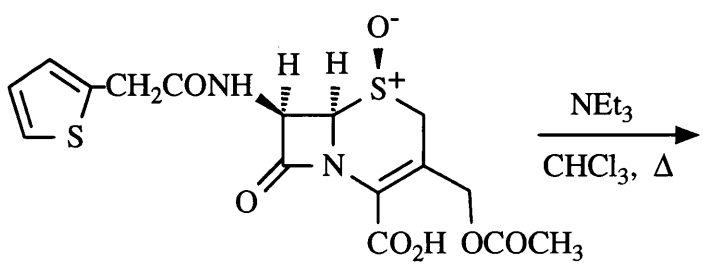
with one exception ie the coupling constant for the C-5 and C-6 protons. Hetacillin shows doublets for these protons with J=4.5 Hz for the *cis* β-lactam whereas epihetacillin shows a coupling constant of J=1.5 Hz indicating a *trans* relationship. To determine which proton had inverted, a deuterium exchange experiment was undertaken. Treatment of hetacillin with sodium deuteroxide followed by deuterium chloride afforded deuteroepihetacillin in 79% yield. The nmr spectrum showed the absence of the C-6 proton doublet and a singlet at δ5.42 ppm replaced the C-5 doublet indicating that H-6 is the more acidic and has undergone epimerisation.

Furthermore they reported[170] that epimerisation also occurs in non-aqueous media. Methyl hetacillin **(396)** epimerised to methyl epihetacillin **(397)** after 5.5 hour in DMSO with triethylamine.

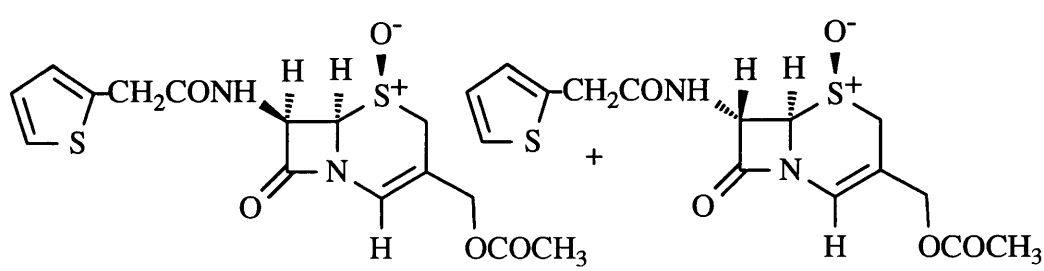


(396) **(397)**

A similiar phenomenon was reported[171] with cephalosporins. Decarboxylation of sulphoxide **(398)** in refluxing chloroform with one equivalent of triethylamine resulted in the formation of two products **(399)** and **(400)** in a 1:4 ratio. Isomerisation of **(399)** to **(400)** was completed with triethylamine in DMSO



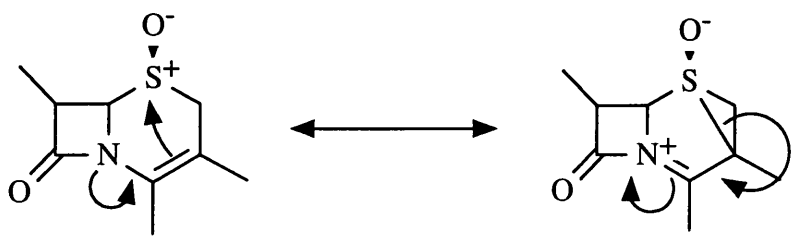
(398)



(399) **(400)**

at 50°C for 48 hours. Again the *trans* structure was assigned on the basis of the decreasing coupling constant from 5 Hz to 2.5 Hz and as before deuterium exchange confirmed the epimerisation of C-7 proton.

The authors postulate[172] why cephalosporins and their respective sulphoxides tend to be more reluctant to epimerise than their penicillin counterparts even although a comparable mechanistic pathway exists. From nmr dilution and solvent studies, it was discovered that amide protons of ceph-3-em sulphoxides are more weakly hydrogen bonded to the β-sulphoxide oxygen than their respective penams, indicating a lower $S^{+}->O^{-}$ dipole moment. In turn this reduces the acidity of the C-7 hydrogen and decreases the chance of enol formation of the β-lactam carbonyl. These effects have been attributed to resonance structures as shown in Scheme 13.



Scheme 13

This tendency may explain why epimerisation was only observed with the Michael addition involving 2-chloroacrylonitrile. The difference between the reactions was that the sulphoxide (**202**) was dissolved in acetonitrile with 2-chloroacrylonitrile instead of being directly suspended in the Michael acceptor. In an attempt to determine whether or not acetonitrile has any effect on epimerisation, a solution of (**202**) in acetonitrile was stirred in the presence of triethylamine. After 5 days tlc indicated that starting material remained unreacted and the build up of baseline material. A more effective experiment would have been to attempt epimerisation with the 2-cyclopropyl adduct (**382**) rather than the sulphoxide (**202**). However, low yields and difficult separations precluded this approach.

2.1.3 Attempted Michael Additions with Ceph-3-em Sulphoxides

Methyl Propiolate

Methyl propiolate was previously reported[173] to undergo Michael additions with arylamines to afford arylaminoacrylates as shown in Scheme 14. It

$$ArNH_2 \quad + \quad HC \equiv CCO_2CH_3 \longrightarrow Ar\text{-}NH\text{-}CH{=}CHCO_2CH_3$$

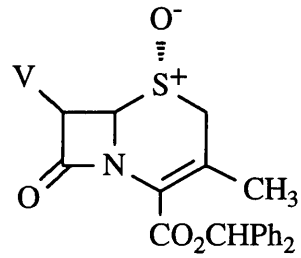
Scheme 14

was therefore considered suitable for a Michael addition with diphenylmethyl ester **(202)**. As in preceding conditions, ester **(202)** was stirred in dry THF with the Michael acceptor in the presence of the triethylamine base at room temperature. After 5 hours, the solvent and base were evaporated under reduced pressure and a black oily residue was obtained which according to ir spectroscopy contained only products of degradation. Modifying the conditions by introducing acetonitrile as the reaction solvent and reducing the temperature to 0°C afforded one β-lactam product in less than 50 mgs after chromatography. As a consequence of the poor yield, the Michael addition of methyl propiolate was not studied further. It is thought that the presence of the triple bond may make the Michael acceptor too reactive resulting in degradation of the β-lactam ring.

3-Ethoxymethacrolein

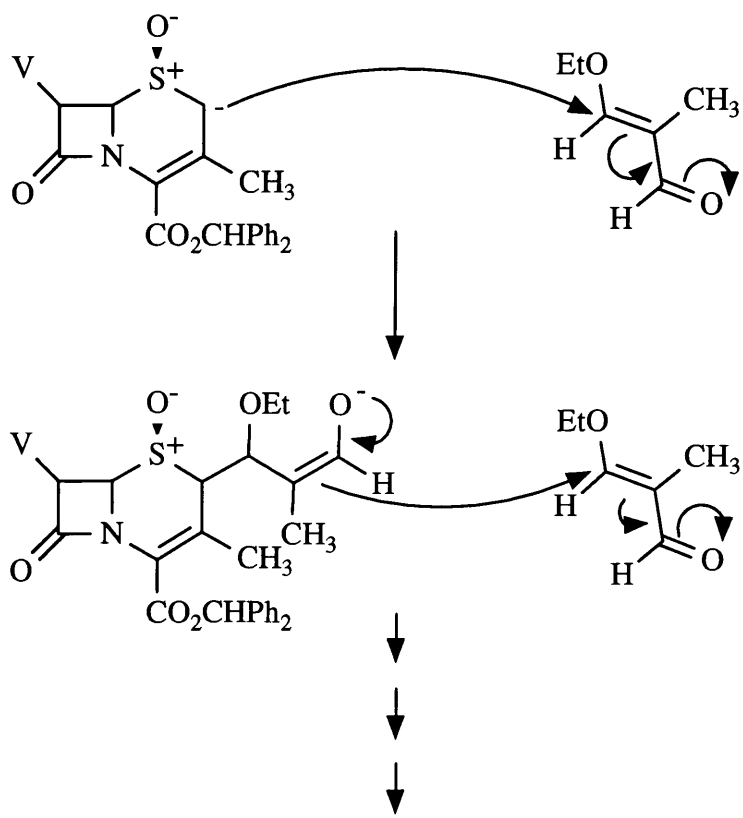
3-Ethoxymethacrolein was also employed as a Michael acceptor but no reaction occurred after 24 hours of stirring in dry THF with the sulphoxide **(202)** and triethylamine. Hence the sulphoxide was directly suspended in 3-ethoxymethacrolein again in the presence of triethylamine and after 24 hours, three products were observed on tlc. However on evaporating the solvents, a gel formed. Attempts to dissolve this gel in ethyl acetate, dichloromethane or acetone were futile. Gels have previously been observed with the R-sulphoxide of the diphenylmethyl ester **(401)** only when it has been a major constituent of a reaction

mixture. In this case it is only present in trace quantities and consequently it is



(401)

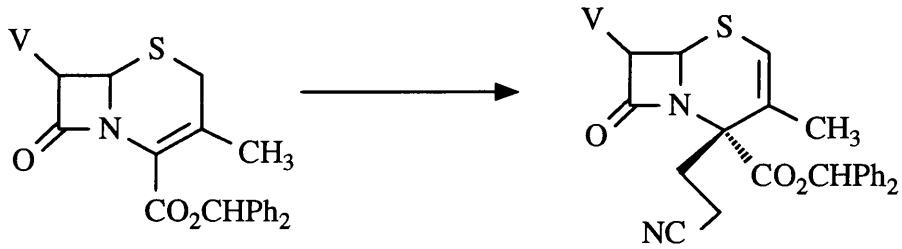
more feasible that the 3-ethoxymethacrolein unit is polymerising as shown in Scheme 15. As a result of these difficulties this reaction was not investigated further.



Scheme 15

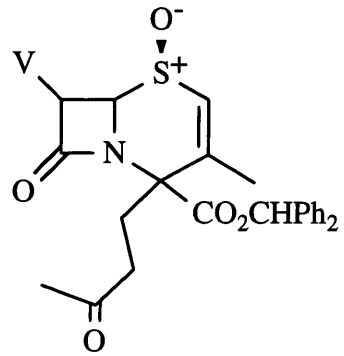
2.1.4 Michael Additions of Ceph-3-em Sulphides

Reaction of the sulphide **(361)** with acrylonitrile results in a 74% yield of the 4-substituted adduct **(362)**[88]. It was therefore considered that if **(362)** was



(361) (362)

oxidised, it might undergo a reverse Michael reaction and produce either the original starting material ie sulphoxide (**202**) or a 2-substituted adduct in a higher yield than obtained from direct Michael addition at the C-2 position. Thus, reaction of the sulphide (**361**) with acrylonitrile in the presence of triethylamine gave one major product as an oil and ir displayed β-lactam (1788 cm^{-1}) and nitrile (2251 cm^{-1}) peaks. The nmr spectrum showed a doublet at δ1.55 integrating for 3 protons with a coupling constant of 1.2 Hz. A signal at δ6.23 for 1 hydrogen, also a doublet with coupling frequency 1.2 Hz, indicated the Δ2-double bond and hence substitution at the C-4 position. A multiplet integrating for 3 protons at δ1.99-2.50 and a multiplet for 1 proton with a chemical shift between δ2.97 and δ3.18 shows the presence of 4 hydrogens coupling with each other and is the result of one molecule of acrylonitrile. The major product is therefore assigned the 4β-substituted structure (**362**) which was afforded in 49% yield. Similiarly Michael addition of the sulphide (**361**) was accomplished with methyl vinyl ketone via a direct suspension of (**361**) in the Michael acceptor with an excess of triethylamine. The C-4 adduct that was generated was oxidised *in situ* in the presence of *m*-CPBA to its corresponding sulphoxide (**385**) in a 74% yield. Spectroscopic and analytical data supported structure (**385**).

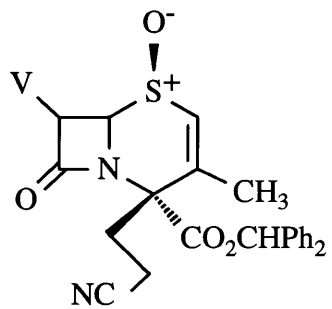


(385)

2.1.5 Oxidation of Michael Adducts

It has been shown by Kaiser *et al*[22] that unsaturated sulphoxides have a preference for β,γ-double bond as opposed to α,β-configuration, and they utilized oxidation as a technique to convert ceph-2-ems into their more thermodynamically stable ceph-3-ems. However, isomerisation from Δ2 to Δ3 unsaturation is not possible under oxidation conditions with 4-disubstituted ceph-2-ems. For example 4-(3'-oxobutyl)ceph-2-em **(44)** is readily oxidised to its corresponding α,β-unsaturated β-sulphoxide **(385)**. It was anticipated that these molecules might undergo a novel retro-Michael reaction.

Before a reverse addition could be attempted the 4-(2'-cyanoethyl) sulphide **(362)** had to be oxidised to its corresponding sulphoxide **(402)** to encourage the formation of a C-2 nucleophile.

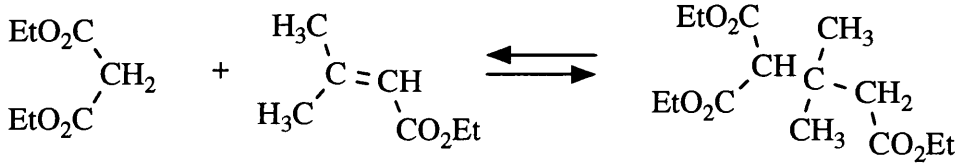


(402)

In the presence of *m*-CPBA for 1 hour **(362)** was converted into **(402)** in 87%. Confirmation of the structure of **(402)** was obtained from the nmr spectrum which was similiar to **(362)** with the exception of the C-2, C-6, C-7 protons and N-H for which the chemical shifts appeared further downfield as expected[174].

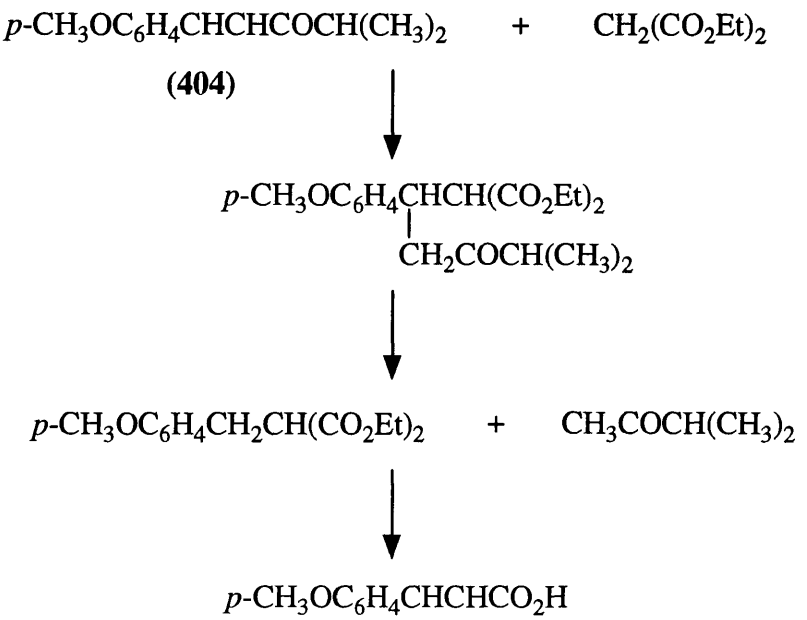
2.1.6 Attempted Retro-Michael Additions

The Michael reaction is a reversible process[175] in which the products can be converted back into the reactants by the same catalysts. Although few publications are available it is apparent that low temperature favours the formation of Michael adducts while higher temperatures promote retrogression. In addition, the reversible reaction is more likely to occur if the condensation process is slow. These two effects are demonstrated in the reaction of diethyl malonate with ethyl 3,3-dimethylacrylate (Scheme 16). At 25°C the yield of adduct **(403)** is 70% and at 100°C the yield is reduced to 30%.



(403)

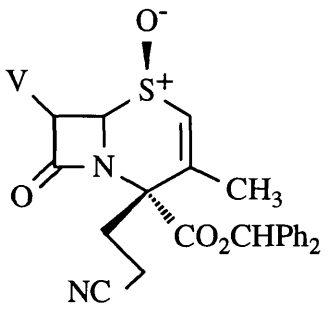
If a Michael condensation product has the symmetrical structure of a 1,5-diketopentane with hydrogen atoms in the 2 and 4 positions, then the reverse reaction can occur to give fragments that differ from the original starting materials eg the Michael reaction of iso-propyl *p*-methoxybenzylidenemethyl ketone **(404)** with diethyl malonate in ethanol results in *p*-methoxycinnamic acid after hydrolysis[176] (Scheme 17).



Scheme 17

Both 4-substituted and 2,2-disubstituted Michael addition products were considered capable of undergoing a reverse Michael reaction to produce the corresponding unsubstituted or 2-monosubstituted adduct. If successful, it would have provided an alternative route to the desired compounds **(368)** and **(384)** which were otherwise produced in poor yields from the usual Michael addition reactions (see Section 2.1)

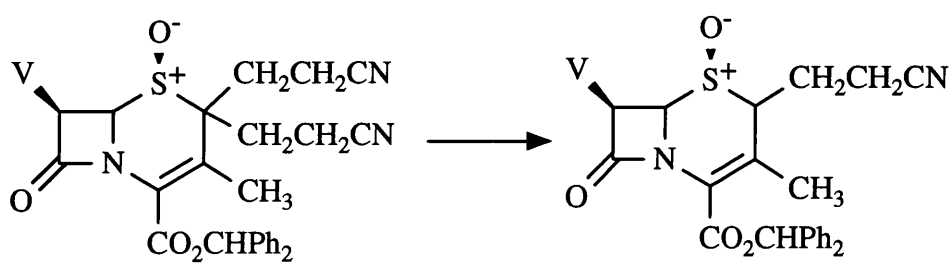
Thus an attempt to reverse the Michael addition reaction was undertaken with the 4-(2'-cyanoethyl) sulphoxide adduct **(402)**. Initially **(402)** was stirred in



(402)

dry THF with excess triethylamine, but no reaction occurred after 24 hours according to tlc. The starting material also remained present when the reaction mixture was heated at reflux for 3 hours. After 24 hours in the presence of the stronger base sodium hydride, some sulphoxide had reacted to give a product slightly less polar on tlc. According to ir, a strong signal for the β-lactam ring was evident at 1777 cm^{-1}. However numerous attempts at separation by column chromatography failed and the structure of this compound remains unknown.

Similiar reactions were endeavoured with the 2,2-di-(2'-cyanoethyl) adduct **(373)** in the hope that one mole of acrylonitrile would be released to form the mono-adduct **(368)** - a compound which proved difficult to obtain from the Michael addition reactions. Likewise, the di-adduct dissolved in dry THF, was



(373) **(368)**

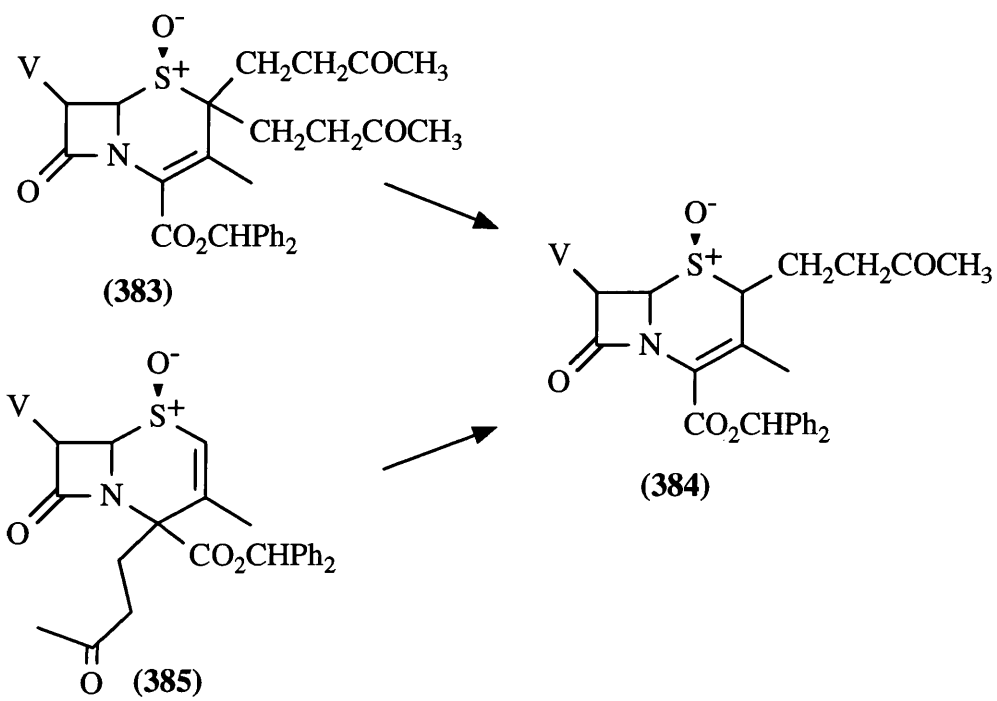
stirred for 24 hours with 3 mole equivalents of triethylamine and according to tlc the starting material remained unreacted. An excess of triethylamine was added and after 24 hours tlc indicated **(373)** was still present, hence the reaction solution was heated at reflux for 2 hours. After this time tlc indicated that the di-adduct had disappeared from the reaction mixture but ir spectrum of the residual brown oil revealed the lack of a β-lactam carbonyl signal and hence the presence of degradation products.

An attempt at retro-Michael addition of **(373)** with sodium hydride as a stronger base in dry THF for 4 hours gave numerous products which proved to be inseparable by chromatography. Di-adduct **(373)** was dissolved in DMF under a blanket of nitrogen at 0°C and stirred with sodium hydride for 24 hours. Although

the di-adduct had reacted, the brown oil obtained on work up contained no β-lactam products (ir).

Finally **(373)** was dissolved in acrylonitrile and stirred in the presence of 3 molecular equivalents of triethylamine for 48 hours. Chromatography afforded three products all resulting from degradation of the β-lactam ring.

Corresponding results were obtained when similiar reactions were carried out with the methyl vinyl ketone Michael addition products. Retro-Michael reactions were attempted with both the 4-mono- **(385)** and 2-di-adducts **(383)** in the hope of producing either the sulphoxide **(202)** or the 2-mono-adduct **(384)**.



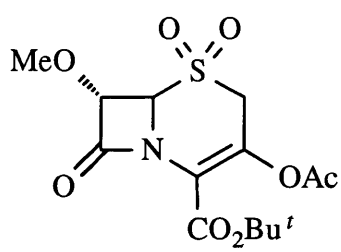
The sulphoxide **(385)**, dissolved in dry THF, was stirred in the presence of triethylamine at room temperature. However, after stirring overnight, tlc indicated no reaction had taken place. Heating at reflux for 3 hours also proved fruitless. As with the 4-(2'-cyanoethyl)ceph-2-em **(402)**, the 4-(3'-oxobutyl)ceph-2-em would not undergo a reverse reaction therefore an attempt to react the 2,2-(3'-oxobutyl) adduct **(383)** was considered. Utilizing

toluene as solvent, the di-adduct was refluxed for 4 hours with an excess of triethylamine and according to tlc starting material remained unreacted. Replacing the triethylamine base with sodium hydride and heating the solution at reflux for 1 hour resulted in the production of baseline material and hence was investigated no further. Modifying the conditions and using the acid catalyst *p*-toluene sulphonic acid in place of triethylamine, cephem **(383)** was refluxed in toluene for 1 hour. However numerous products were observed on tlc (as well as baseline material).

As with the attempted retro-Michael reactions of 2'-cyanoethyl adducts, either no reaction occurred or numerous products were afforded which were inseparable on silica gel, therefore further studies in this area were not continued.

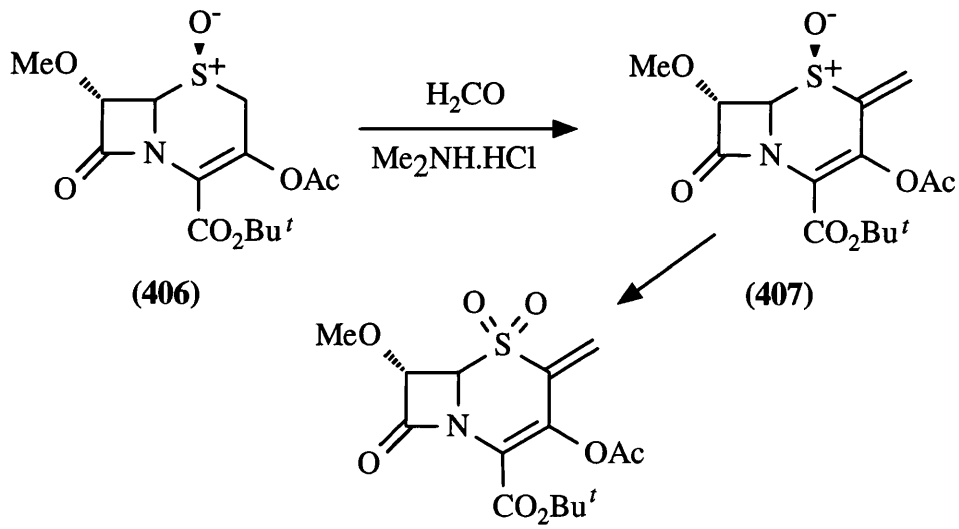
2.1.7 Attempted Michael Additions of Ceph-3-em Sulphones

Human Leucocyte Elastase (HLE) is an enzyme that is implicated in the tissue destruction attributed with pulmonary emphysema[177]. A recent publication reported that a number of cephalosporin sulphones with C-2 substituents were tested as inhibitors of HLE. These substituents gave a considerable increase in activity against this enzyme over their corresponding unsubstituted parent **(405)**.

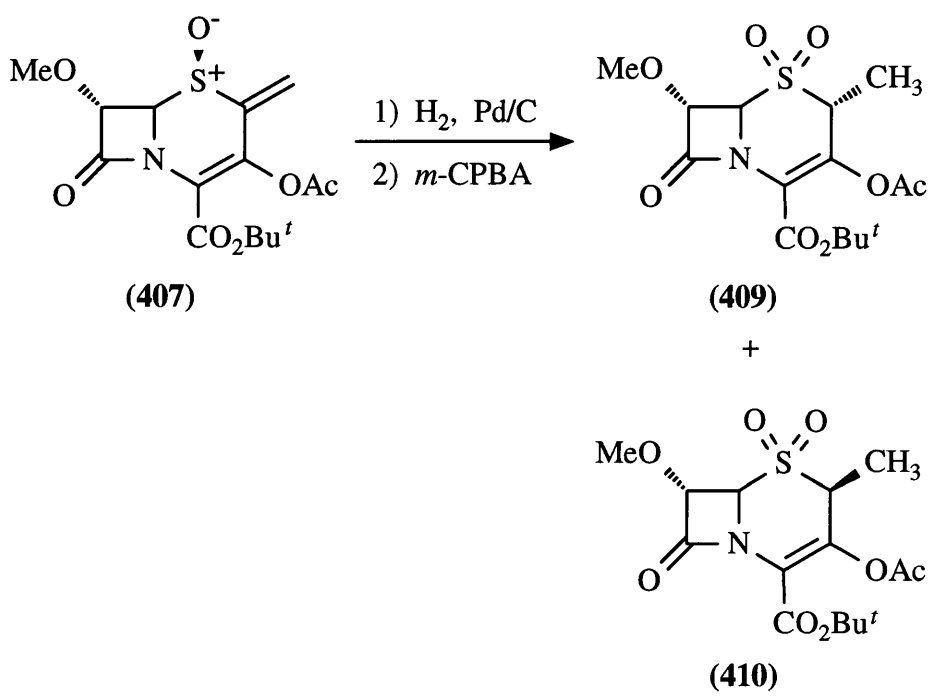


(405)

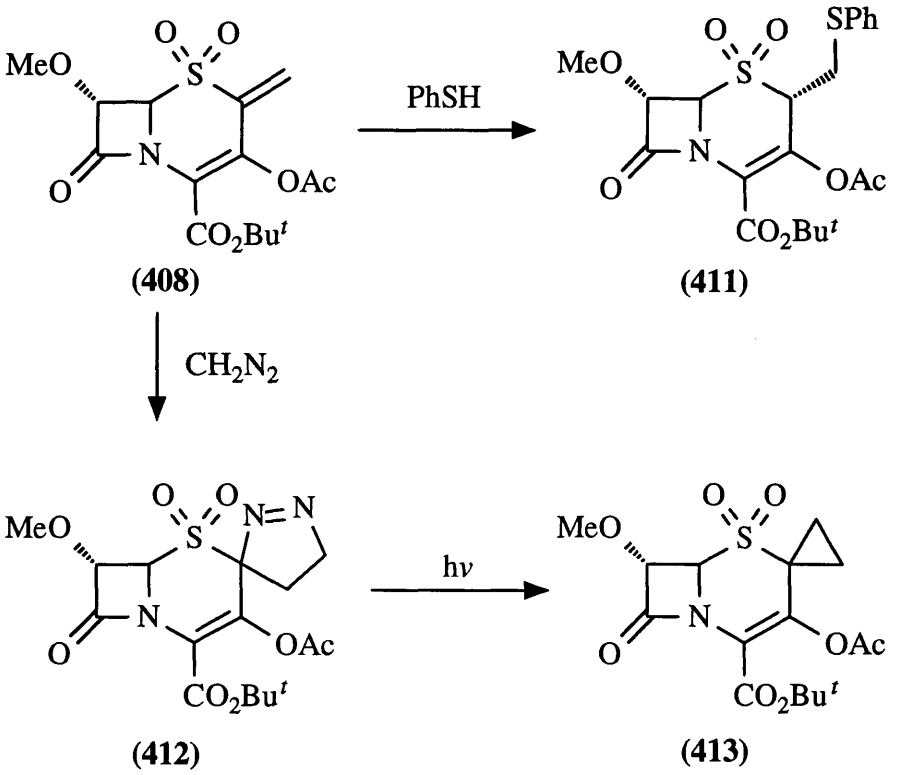
These derivatives were synthesised by reaction of the corresponding β-sulphoxide **(406)** under Mannich conditions with formaldehyde and dimethylamine hydrochloride to afford the 2-methylene ceph-3-em sulphoxide **(407)**. Oxidation of **(407)** with *m*-CPBA gave the sulphone **(408)**.



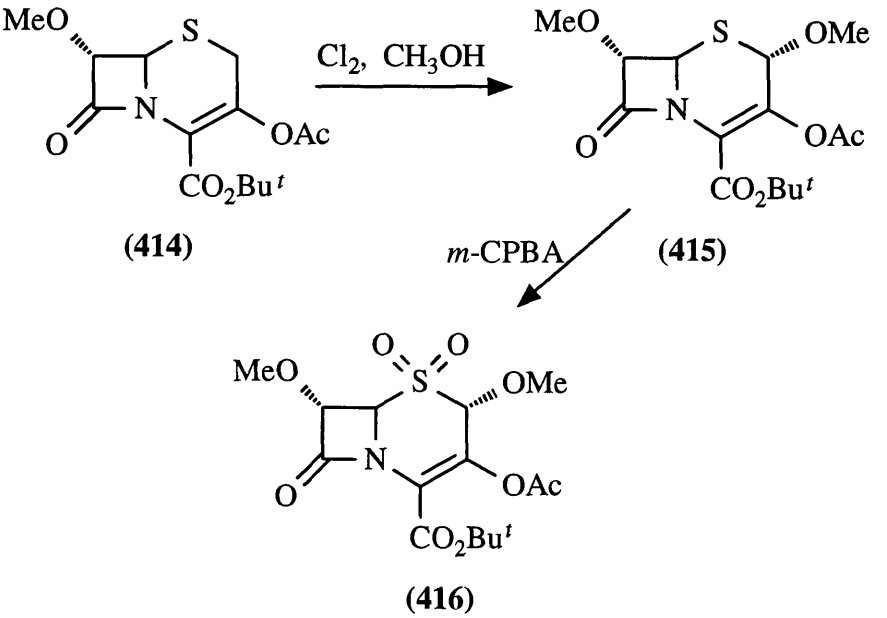
Hydrogenolysis of **(407)** followed by oxidation gave the α- and β-methyl sulphones **(409)** and **(410)**.



Treatment of **(408)** with thiophenol and diazomethane gave **(411)** and **(412)** respectively. The spiropyrazoline **(412)**, highly reactive in light or heat conditions, lost nitrogen and afforded the 2-spirocyclopropylceph-3-em **(413)**.



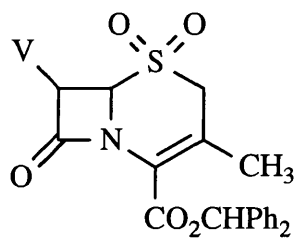
Reaction of the sulphide **(414)** with excess chlorine in methanol afforded the 2α-methoxy ceph-3-em **(415)**. Oxidation with excess *m*-CPBA gave the sulphone **(416)**.



Sulphones **(408)**, **(409)**, **(410)**, **(411)** and **(416)** all displayed increased inhibitory activity against HLE compared to the parent sulphone **(405)**.

Consequent to this report various other HLE inhibitors were published including C-7 substituted ceph-3-em sulphones[178], C-4 amide substituted sulphones[179] and monobactams[21].

As a result of a 2-exomethylene bond being formed directly from the Michael addition of diethyl ethoxymethylenemalonate to the sulphoxide **(202)**, it was considered a model reaction for the synthesis of a potential HLE inhibitor using the sulphone **(417)**. Thus, **(413)** was stirred overnight in the Michael



(417)

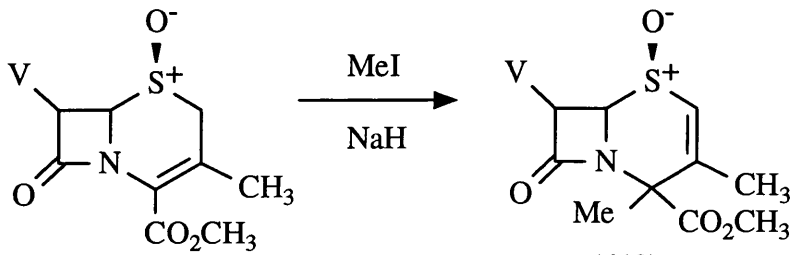
acceptor in the presence of triethylamine after which time tlc indicated reaction had gone to completion. However ir spectroscopy showed the products were the result of degradation of the β-lactam ring and so the reaction was not investigated further.

In all probability a more successful preparation would have been to oxidise the corresponding 2-substituted sulphoxide **(390)** over a direct Michael addition with the sulphone but such low yields and difficult separations precluded this approach.

2.2 Reactions with Haloalkanes

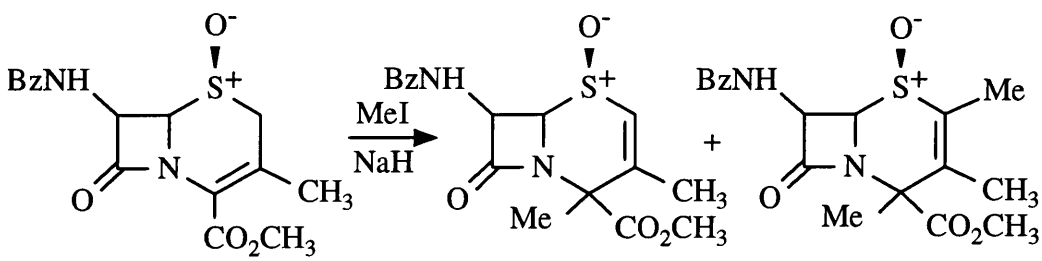
Previous research has seen the successful alkylation at C-2 and C-4 of ceph-3-ems using a variety of electrophiles and as addition of Michael acceptors to the C-2 position was not as fruitful as expected, it was considered that reaction with haloalkanes would provide the desired 2-monoalkylated ceph-3-ems.

Dolfini[180] investigated these reactions in 1972 using alkali metal hydrides, hydroxides and alkoxides as bases. Thus reaction of the ceph-3-em β-sulphoxide **(418)** with iodomethane in the presence of sodium hydride at 0°C for 2.5 hours resulted entirely in **(419)**. Under the same conditions, using the



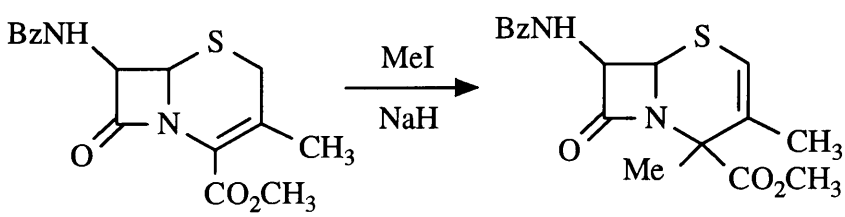
(418) **(419)**

ceph-3-em **(420)**, Yoshida *et al*[37] reported 74% of the 4-methylceph-3-em **(421)** and also observed the 2,4-dimethylceph-3-em **(422)** in 9.5% yield. Using the



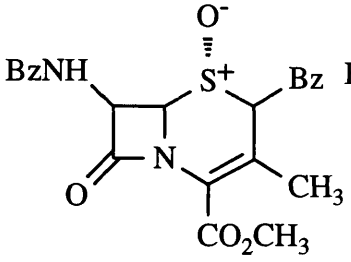
(420) **(421)** **(422)**

corresponding sulphide **(423)** and lithium diisopropylamide as the base, the Sankyo workers[23] observed the C-4 alkylated ceph-3-em **(424)** in a yield of 55%.



(423) **(424)**

Employing benzyl bromide as the electrophilic reagent with sodium hydride base, the 2-benzylceph-3-em **(425)**, 2,4-dibenzylceph-3-em **(426)** and the 4-benzyl ceph-2-em **(427)** were isolated in 27%, 25% and 25% yields respectively.



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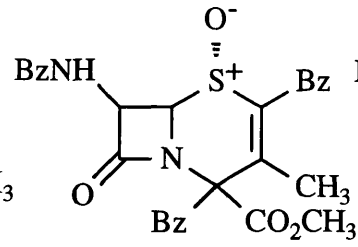

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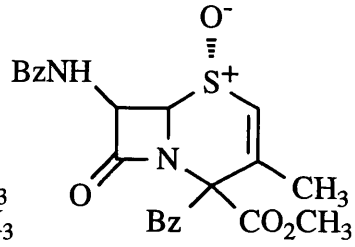
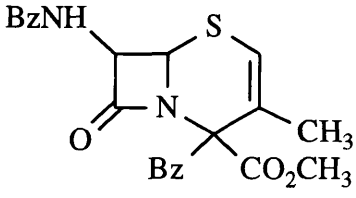
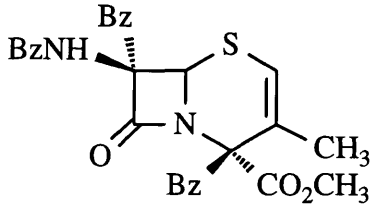


(427)

Benzyl bromide also reacted with the sulphide and lithium diisopropylamide to give a mixture of the 4β-mono and 4β,7α-disubstituted ceph-2-ems **(428)** and **(429)** respectively.



(428)



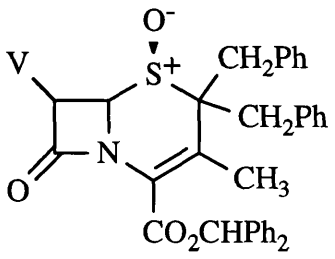
(429)

2.2.1 Alkylation of Ceph-3-em Sulphoxides

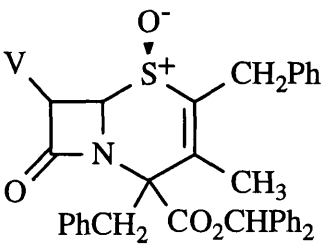
Benzyl Bromide

In this work when benzyl bromide was stirred with β-sulphoxide **(202)** and sodium hydride in DMF at 0°C, two products with similiar Rf's were observed on tlc but they remained inseparable after numerous attempts at chromatography. Modifying the solvent to DMSO and adding a solution of **(202)** in DMSO dropwise to sodium hydride in DMSO also resulted in a complex mixture of products. The numerous compounds produced were a direct result of the reaction being carried out at room temperature to accommodate the solvent. Dolfini[179] advised that this type of reaction should preferably be carried out at temperatures of between -10°C and 10°C. Thus, DMF was again used as solvent and sodium hydride was added in two portions over a period of 30 minutes to a stirred suspension of the sulphoxide **(202)** and benzyl bromide in DMF. One

component which was isolated by chromatography displayed the desired β-lactam carbonyl (ir 1780 cm^{-1}). The di-adduct structure was confirmed from the nmr spectrum, where a multiplet appeared for the two -CH_2 groups of the benzyl and an extra two phenyl groups were observed at δ6.91-7.44. However, two possible structures exist for the di-adduct ie the 2,2-disubstituted ceph-3-em **(430)** and the 2,4-disubstituted ceph-2-em **(431)**.



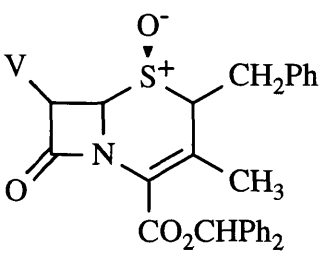
(430)



(431)

The Sankyo workers[37] reported two singlets for the -CH_2Ph groups of their 2,4-dibenzyl adduct where as Ringan[181] observed two AB quartets. Thus, as a result of the complex multiplet it is hypothesised that the benzyl groups must be in close environments resulting in coupling between all 4 protons and hence structure **(431)** is assigned. Elemental analysis and mass spectroscopy support both structures but further experimental work confirmed the compound as the 2α,4β-dibenzylceph-2-em **(431)** (see Section 2.6).

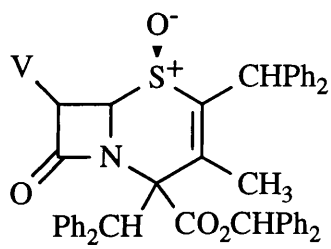
The yield of 10% was calculated from isolated product but a mixture of 2.49g of di-adduct **(431)** and another component remained inseparable. The contaminating product was assumed to be the desired 2-benzyl ceph-3-em **(432)** and a Pummerer rearrangement was attempted on this mixture (see section 2.3.1).



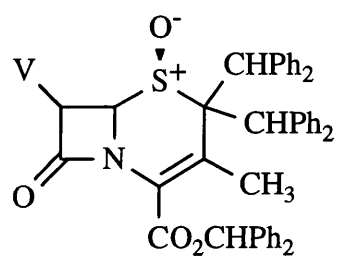
(432)

Bromodiphenylmethane

Employing bromodiphenylmethane as the electrophilic reagent in place of benzyl bromide, the substitution required 15 hours in DMF with sodium hydride and the reaction mixture was cleaner as expected. Two major products were isolated of which the least polar had incorporated two diphenylmethyl groups, as indicated by the large increase in the integration figure for the multiplet at δ6.73-7.5. Also two singlets at δ5.15 and δ5.38 integrating for one proton each was symptomatic of two $CHPh_2$ groups. The remainder of the spectrum was in accord with the cephem nucleus and thus, the possible structures were either the 2,4-di-diphenylmethylceph-3-em **(433)** or the 2,2-di-diphenylmethylceph-3-em **(434)**.



(433)

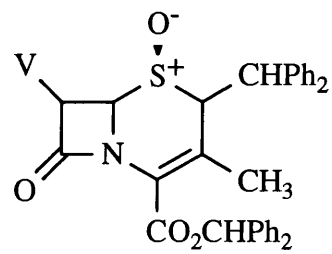


(434)

However, it was thought not possible for the two bulky diphenyl groups to exist at C-2 and at this point the di-adduct was assigned the structure **(433)**. Further confirmation on this structure was obtained from elemental analysis which indicated a molecular formula of $C_{55}H_{46}N_2O_6S$ and from mass spectroscopy which displayed a molecular ion at 863 for MH^+. Further experimental work to confirm this structure is detailed in Section 2.6.

The second isolated product also displayed an increase in integration figure for the multiplet at δ6.9-7.4, however not as large as the di-adduct, and it was concluded that only one diphenylmethyl group was incorporated. In addition a doublet at δ3.93 with coupling constant J=8.5 Hz and a doublet at δ4.39 with a

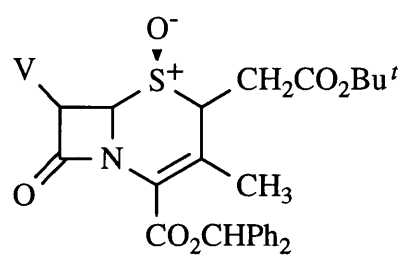
similiar coupling constant represented the C-2 proton and the proton from the diphenylmethyl group also attached at C-2. Verification of the ceph-3-em structure was obtained from the C-3 methyl group which was observed in the spectrum as a singlet. The mass spectrum gave two molecular ions ie 697 for MH^+ and 714 for MNH_4^+ indicating a molecular weight of 696 amu which would satisfy the formula $C_{42}H_{36}N_2O_6S$, again indicating the addition of one diphenylmethyl group. Therefore from the above information the most polar product was assigned to the 2-diphenylmethylceph-3-em structure **(435)** which was obtained in a 26% yield, slightly higher than the yield of the di-adduct **(431)** which was 21%. Again microanalysis figures supported this structure.



(435)

<u>*t*-Butyl Bromoacetate</u>

When the diphenylmethyl ester **(202)** was reacted with *t*-butyl bromoacetate, exclusive formation of the 2-(*t*-butyloxycarbonyl)methylceph-3-em **(436)** was reported[181]. Hence, with the previous separation problems experienced,



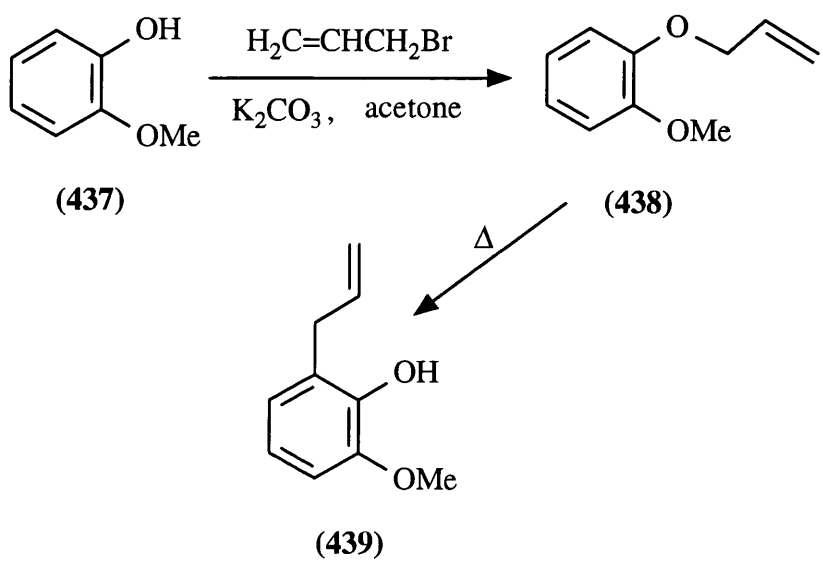
(436)

t-butyl bromoacetate was considered a good electrophile which would give a desired 2-alkylated ceph-3-em relatively easily. Thus reaction of **(202)** with this

electrophile was accomplished in 1 hour in the presence of sodium hydride base using DMF as solvent. One major component, isolated by column chromatography, contained the β-lactam ring according to ir (1801 cm^{-1}). Spectroscopic data were similiar to the previously prepared material eg a singlet integrating for 9 protons at δ1.27 signified the *t*-butyl group, three double doublets appearing at δ2.01, δ2.42 and δ3.80 indicated the 2-H and the $CH_2CO_2Bu^t$. As expected alkylation has occurred at C-2 and the structure **(436)** was assigned. Compared to preceeding reaction yields, **(436)** was obtained in a reasonably good yield of 48%, it was low however compared to that reported earlier[181].

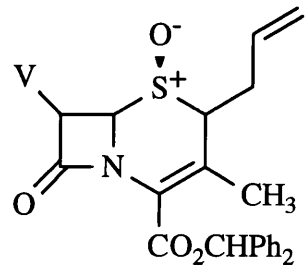
Allyl Bromide

Allyl bromide has been widely used as an electrophilic source of the allyl group in alkylations. The O-alkylation of **(437)** to **(438)** in 80-90% has been described[182] and reflux of product **(438)** gave a high yield (80-90%) of the Claisen rearrangement product **(439)**.



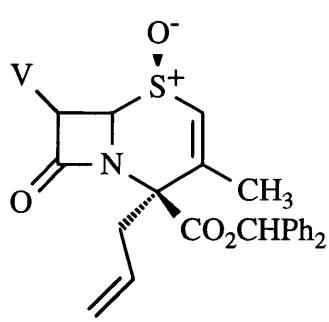
(437) **(438)** **(439)**

Therefore it was anticipated that reaction of allyl bromide with the sulphoxide **(202)** would produce the 2-alkylated ceph-3-em **(440)**. Thus, **(202)** was



(440)

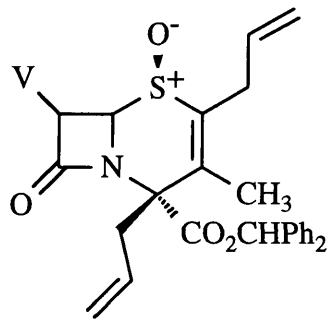
stirred overnight at 0°C under a blanket of nitrogen, with allyl bromide in the presence of sodium hydride using DMF as solvent. After chromatography two products were isolated and spectroscopic data of the major compound gave an unusual signal at δ6.7 integrating for 1 proton. The signal is typical for a 2-H of a ceph-2-em. However it usually appears as a doublet due to vinylic coupling with the 3-CH_3. In this case no coupling was apparent. Additionally the upfield multiplets at δ2.85-2.95 and δ3.82-3.88 integrating for one proton each signify $C\underline{H}_2CH=CH_2$; a multiplet with a chemical shift from δ5.14 to δ5.2 integrating for 2 protons indicates $CH_2CH=C\underline{H}_2$, and a multiplet downfield at δ5.93-6.01, again integrating for 1 proton signifies the $CH_2C\underline{H}=CH_2$ proton and therefore the incorporation of one allyl group. The remainder of the spectrum is in accordance with the ceph-2-em nucleus and the C-4 alkylated structure **(441)** was tenously assigned. Both mass spectroscopy and elemental analysis supported this structure.



(441)

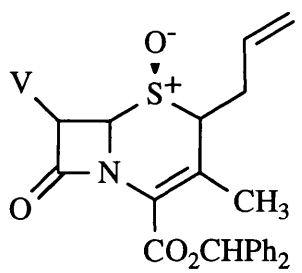
The number of protons present in the nmr spectrum for the minor product indicated the addition of two allyl groups to the sulphoxide **(202)**.

Furthermore the signals for the two CH_2 groups adjacent to the double bond in each allyl group ($C\underline{H_2}CH{=}CH_2$) are different. One signal appears as a multiplet at δ3.24-3.27 integrating for 2 protons while the other is observed as two multiplets at δ2.83-2.93 and δ3.81-3.89 both integrating for 1 proton each. Consequently these different signals suggest that the allyl groups are in different environments and the di-adduct was assigned the 2,4-alkylated configuration as shown by **(442)**.



(442)

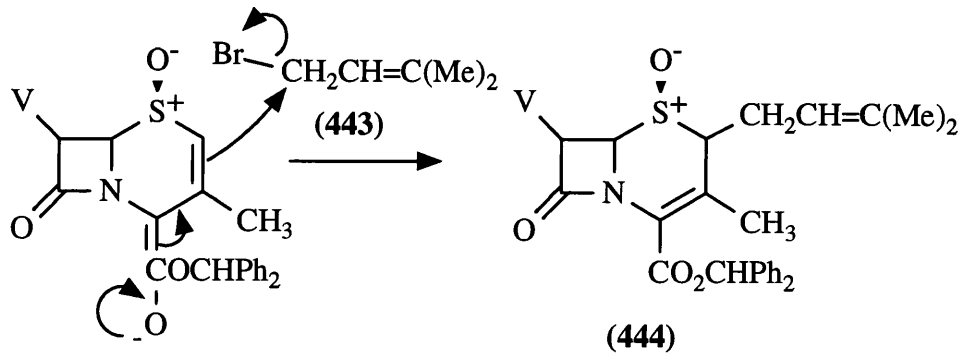
Although other products were present in the reaction mixture, only **(441)** and **(442)** were isolated in 24% and 13% yields respectively and the desired 2-alkylated product **(440)**, if present, remained elusive.



(440)

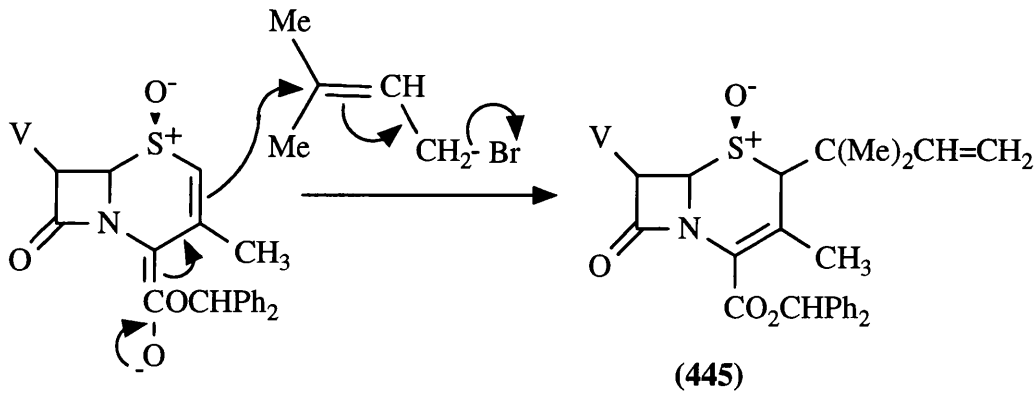
1-Bromo-3-methylbut-2-ene

The electrophile 1-bromo-3-methylbuten-2-ene **(443)** should have similiar reactions to allyl bromide and therefore it was considered for reaction with the sulphoxide **(202)**. If reaction occurred at the C-2 position then two possible pathways of alkylation can exist. As shown in Scheme 18 the sulphoxide anion



Scheme 18

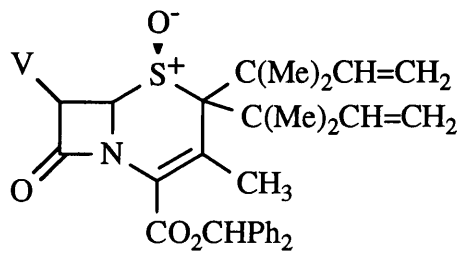
could attack the first carbon displacing a bromide ion to give **(444)** or alternatively nucleophilic attack occurring at the third carbon, displacing the double bond and eliminating bromide (Scheme 19) to afford **(445)**. In the presence of sodium



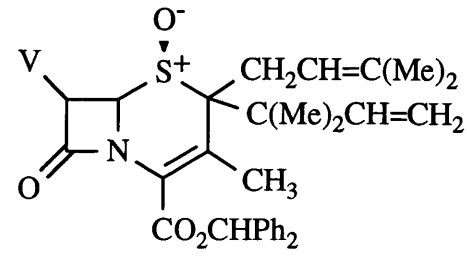
Scheme 19

hydride at 0°C, neither of the above substitutions appeared to take place and after 15 hours excess sodium hydride and 1-bromo-3-methylbut-2-ene were added. Within 3 hours of stirring at room temperature numerous products were observed on tlc of which only one could be isolated via column chromatography. Integration of the nmr spectrum for this major product indicated the presence of an extra 18 protons, suggesting the incorporation of two molecules of the electrophile and again the formation of a di-adduct.

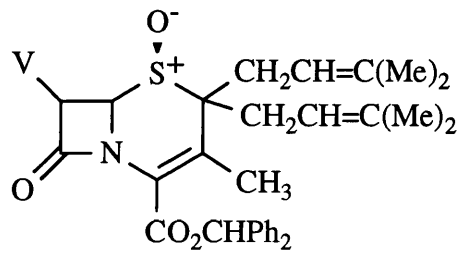
Considering the 2,2-di-adduct for the moment then three possible isomers exist ie **(446)**, **(447)** and **(448)**. For structures **(446)** and **(447)**, the protons



(446)



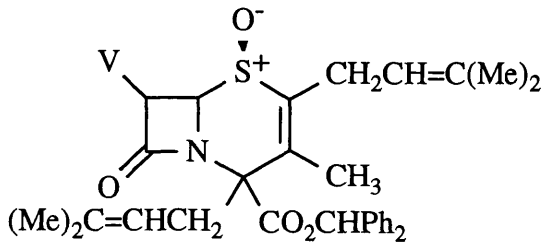
(447)



(448)

from the CH_2 groups attached to the double bond would appear at δ5.0. However the nmr shows 4 protons as a multiplet between δ2.95 and δ3.62 ie too far upfield for them to be of an unsaturated system. Additionally the spectrum shows the four methyl groups as two singlets at δ1.60 and δ1.71 both integrating for 6 protons and multiplet signals at δ4.92-5.02 and δ5.29-5.31 integrating for 1 proton each for the CH groups of the unsaturated systems. From the spectrosopic data, the most feasible structure was **(448)**. Considering both mechanistic pathways (Scheme 18 and 19), Scheme 18 is more likely to occur as the two methyl groups do not hinder the nucleophilic attack.

There is also the possibility of a 2,4-di-adduct which would have the structure **(449)** and similiar spectroscopic data and elemental analysis as the 2,2-di-adduct. Thus, it is impossible to distinguish between **(448)** and **(449)** and since neither structure was of synthetic value in this work the reaction was not investigated further.

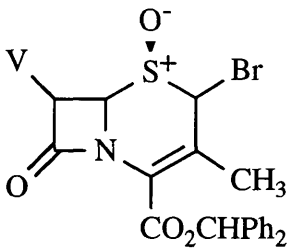


(449)

On account of the complex reaction mixture from reaction of **(202)** with 1-bromo-3-methylbut-2-ene and the isolation of only the di-adduct, no further investigations on this haloalkane were carried out.

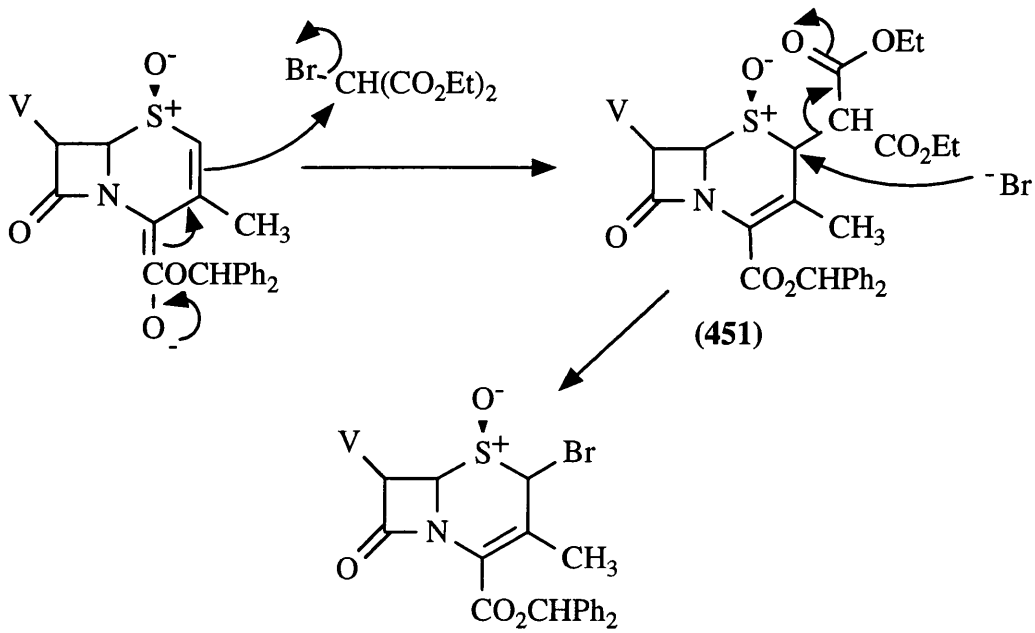
Diethyl Bromomalonate

When using diethyl bromomalonate as the electrophilic reagent, one major product was isolated as an oil after 45 minutes in the presence of sodium hydride base at 0°C. The β-lactam ring was intact according to ir spectroscopy (1801 cm^{-1}) and nmr gave the expected spectrum for a ceph-3-em nucleus. Only one C-2 hydrogen was observed at δ4.75 as a singlet and no extra hydrogens were present, therefore it is concluded that substitution has occurred at C-2 with a species that does not contain any hydrogens. Thus, it was speculated that the major product was the 2-bromoceph-3-em (450) which was afforded in 31% yield.



(450)

A possible mechanism is attack of the diethyl bromomalonate by the sulphoxide anion causing the elimination of a bromide ion and this ion has in turn attacked the C-2 displacing the diethyl malonate (Scheme 20).



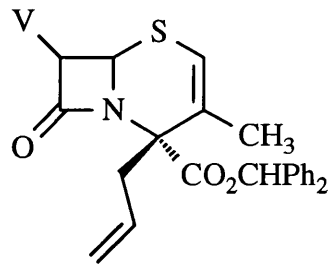
Scheme 20

As the reaction mixture contained numerous products it is possible that some of desired **(451)** is present and has not undergone a second substitution. However, several attempts at column chromatography failed to eparate any other products and, as **(450)** was not required, the reaction was not investigated further.

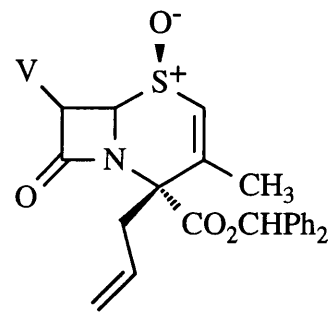
2.2.2 Alkylation of Ceph-3-em Sulphides

Alkylation of the sulphide **(361)** with allyl bromide resulted in the 4-alkylated ceph-2-em **(452)** - a 1,5-diene which has the potential to undergo a Cope rearrangement. The product from a successful Cope rearrangement would be a C-2 monosubstituted derivative which has proved difficult to prepare by other means. Therefore to produce more of the 4-adduct, the sulphide was treated with allyl bromide in the presence of sodium hydride under similiar conditions. One major product was produced and identified as a Δ2 isomer due to the familiar 2-H and 3-CH_3 vinylic coupling from nmr. Also a multiplet integrating for two hydrogens which appeared upfield compared to the other protons from the allyl

system was therefore assigned to the protons adjacent to the double bond ie $C\underline{H}_2$-CH=CH_2. The complex signal was the result of long distance coupling. The signals for the protons of the unsaturated system were complicated multiplets which appeared downfield. Both the integration of the number of protons from nmr and the mass spectrum indicate only one alkylation has taken place and because of the 2-H and 3-CH_3 coupling, alkylation must have occurred at the C-4 position. Thus the major product was assigned structure (452).



(452)



(453)

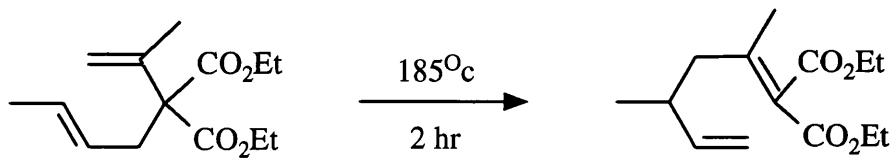
Oxidation of **(452)** with *m*-CPBA resulted in the sulphoxide **(453)** which displayed the usual differences in its nmr spectrum when compared to the sulphide. These differences have previously been reported[174] and are the downfield shifts of N-H, 7-H, 2-H, and the upfield shift of 6-H.

It is worth noting that vinylic coupling between the C-2 proton and the C-3 methyl group was observed in the nmr spectrum of the product **(452)**. Furthermore the coupling is still present in the spectrum for the sulphoxide **(453)** unlike the 4-adduct from reaction of the sulphoxide **(202)** and allyl bromide alkylation. Nevertheless apart from this one small inconsistency both spectra are identical.

2.2.3 Attempted Isomerisation from C-4 to C-2 via a Cope Rearrangement

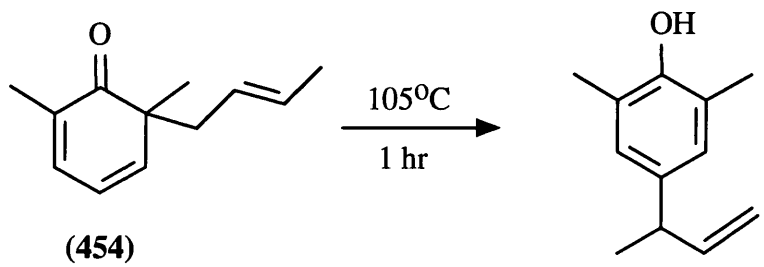
In general 1,5-dienes isomerise in a sigmatropic rearrangement when heated[183]. This has been termed the Cope rearrangement. Cope and co-workers'

original research involved 1,5-hexadiene systems which encouraged rearrangement to occur by providing a driving force of double bond conjugation with groups such as phenyl, carbethoxy or cyano. Hence, rearrangements occurred readily, when the 1,5-hexadienes were heated at temperatures of 150-200^{O}C, in good to excellent yields as shown in Scheme 21.



Scheme 21

Allylated cyclohexadienones such as **(454)** also undergo[184] a Cope rearrangement since the hexadiene system contains an activating group. This rearrangement has an additional driving force of aromatisation and hence less stringent conditions are utilised to convert **(454)** into **(455)**.

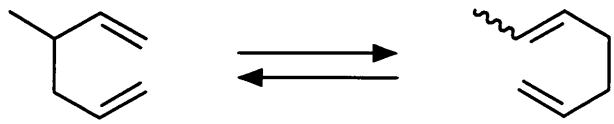


(454) **(455)**

Most Cope rearrangements have been accomplished[184] by heating the neat sample in sealed tubes at temperatures above 150°C. Some rearrangements involve the use of an inert solvent such as cyclohexane, decane and xylene. Furthermore the rearrangement takes place more easily when a conjugating group is present on the third or fourth carbon where the new double bond is formed.

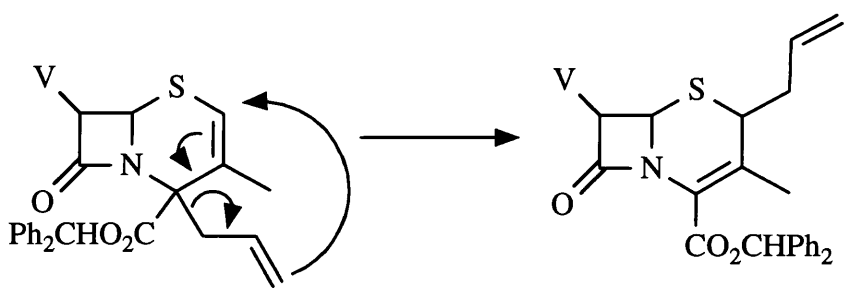
Usually when the 1,5-diene contains only alkyl substituents more stringent conditions are required. The rearrangement may also be incomplete, depending on the comparable stabilities of the isomers. For example, an

equilibrium occurs between 3-methyl-1,5-hexadiene and 1,5-heptadiene at 220-300°C as shown in Scheme 22.



Scheme 22

In this work, reaction between allyl bromide and the sulphide **(361)** furnished the 1,5-diene **(452)** which theoretically should undergo the Cope rearrangement (Scheme 23). Thus, **(452)** was heated at reflux in toluene for 6 hours. No reaction occurred and **(452)** was recovered intact. Using an oil bath and



(452)

Scheme 23

heating **(452)** in xylene at 150-160°C for 1 hour resulted in products due to the degradation of the β-lactam ring (ir).

In an effort to induce the rearrangement, **(452)** was oxidised with *m*-CPBA in dichloromethane to its corresponding sulphoxide **(441)**. After 1 hour the solvent was replaced by toluene and the solution refluxed for 3 hours. A complex reaction mixture was observed on tlc. The sulphoxide **(441)** was purified, dissolved in toluene and refluxed for 5 hours after which time **(441)** was recovered unreacted. As with the sulphide **(452)**, **(441)** was heated at 150-160°C in xylene for 1 hour and ir indicated degradation of the β-lactam ring.

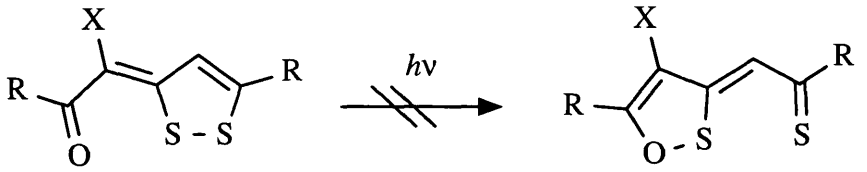
It appears that for the Cope rearrangement to occur, higher temperatures are required, but raising the temperature with these type of molecules results in decomposition.

2.2.4 Regiospecificity of Ceph-3-em Alkylations

Atoms, molecules, ions and free radicals can be classed as either 'hard' or 'soft' Lewis acids and bases[185]. It is stated that acids have a greater affinity for bases in a similiar class eg 'hard' acids have a tendency to react with 'hard' bases and hence form a stronger bond. 'Hard' acids form weaker bonds with soft bases however, and are therefore more reluctant to react. Put simply a hard-hard or soft-soft combination is more stable and more likely.

The softness of a species is dependent on factors such as size, charge, oxidation state, electronic structure and other groups which are attached. A group having a heavier or more electropositive central atom is a softer base within a congeneric series eg $CH_3^- > NH_2^- >^- OH > F^-$. It is also understood that the more carbonium character that a centre attains during a reaction the harder it is. As a result, substitution is often predicted via HSAB theory; eg disulphides RSSR' (soft-soft) are stable where as sulphenyl esters RSOR' (soft-hard) are quite labile.

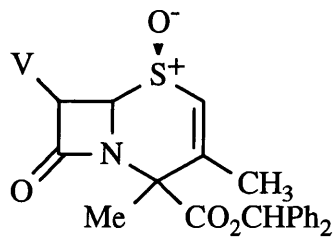
Similiarly the conversion of **(456)** to **(457)** is unfavourable because it involves breaking an S-S bond to form an S-O bond. The acyl group is a hard Lewis acid (RCO^+) hence combination with soft bases results in a highly reactive species.



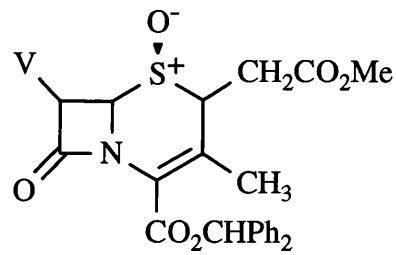
(456) **(457)**

Unpublished work by N Ringan[181] suggests that regiospecificity of alkylation reactions with ceph-3-em sulphoxides is perhaps an indication of the relative hardness or softness of the alkylating agent. It was postulated that the C-4 anion was softer than the C-2 anion because alkylation of sulphoxide **(202)** with iodomethane resulted in exclusive formation of the 4β-methylceph-3-em **(192;**

R=CHPh$_2$ and R^1=Me). On the other hand *t*-butyl bromoacetate, which is assumed to be harder than iodomethane, reacted solely at C-2 to give **(458)** thus revealing C-2 as the harder anion.



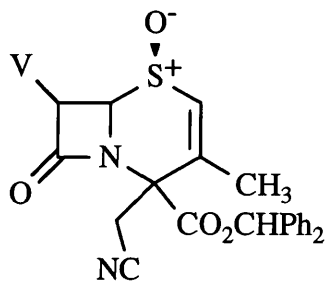
(192)



(458)

In an attempt to confirm this assumption, sulphoxide **(202)** was reacted with chloroacetonitrile, bromoacetonitrile and iodoacetonitrile. From the principles of hardness and softness, chloroacetonitrile would be expected to be the hardest of the three reagents, followed by bromoacetonitrile and iodoacetonitrile would be the softest. Hence, from the unpublished results[181] it would be expected that chloroacetonitrile would alkylate at C-2 and iodoacetonitrile would tend towards C-4 alkylation. Reaction of the sulphoxide **(202)** with chloroacetonitrile was accomplished in 3 hours after an extra addition of sodium hydride and the electrophilic reagent. One product was isolated from column chromatography and ir confirmed the presence of the β-lactam ring (1786 cm^{-1}) as well as the nitrile group (2218 cm^{-1}). The mass spectrum gave a molecular ion of 569 for M^+ and 593 for MNa^+ (an increase in 40 amu) indicating the incorporation of a cyanomethyl group and from nmr the C-3 methyl group appears as a doublet with coupling constant 0.96 Hz showing the presence of a Δ2-cephem structure and therefore the addition of chloroacetonitrile has occurred at the C-4 position. An ABq at δ3.72 and δ3.88 represents the protons from the cyanomethyl group and the rest of the spectrum is in accordance with the diphenylmethyl ester ceph-2-em nucleus. The C-2 proton which should have been a doublet due to vinylic coupling with C-3 methyl was not observed but was included in the multiplet signal for the

ester and the side-chain group. Consequently the product from reaction of **(202)** with chloroacetonitrile was identified as the 4-cyanomethylceph-2-em **(459)** which was obtained in 21%. Starting material **(202)** and an inseparable mixture of **(202)** and **(459)** were also recovered. Additionally microanalysis experimental figures ie



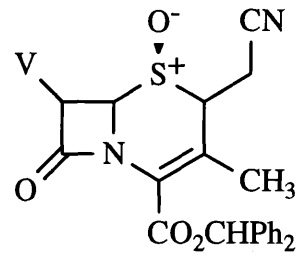
(459)

C,65.22; H,4.72; N,7.39; S,5.53 satisfied the required figures for $C_{31}H_{27}N_3O_6S$ which were C,65.37; H,4.74; N,7.38 and S,5.62.

Reaction of **(202)** with bromoacetonitrile was originally carried out using the same procedure; ie stirring the sulphoxide and the alkylating reagent in the presence of sodium hydride with DMF as the solvent. However after 2 hours, tlc indicated the starting material had been converted into baseline material. The weaker base triethylamine was therefore used with acetonitrile as solvent and after overnight stirring the sulphoxide remained unreacted. Excess triethylamine and bromoacetonitrile were added at room temperature and again after overnight stirring , the build up of baseline material was observed on tlc. Finally stirring **(202)** with bromoacetonitrile in DMF and employing potassium *t*-butoxide, two products were present according to tlc. Both components were isolated by chromatography and the major product was identified as the 4-cyanomethylceph-2-em **(459)** by identical spectra to the product produced from **(202)** and chloroacetonitrile.

The second product exhibited both the β-lactam and cyano signals on ir (1795 and 2250 cm^{-1} respectively). Furthermore it displayed the same molecular ion 593 for MNa^+ implying the incorporation of one cyanomethyl group. From

nmr the C-3 methyl appears as a singlet indicating the Δ3-double bond and two doublets at δ3.04 and δ3.21, both integrating for one proton but with different coupling constants ie 9.6 and 4.3 hz signify the CH_2CN protons. Both hydrogens are split by the C-2 proton which is a double doublet at δ4.35 and have the same coupling constants. Thus the minor product was assigned the structure **(460)** which was supported by elemental analysis. The 4-cyanomethylceph-2-em **(459)**



(460)

was produced in 31% yield and the 2-cyanomethylceph-3-em **(460)** was afforded in 8%. Reaction of the sulphoxide **(202)** with iodoacetonitrile was attempted with potassium *t*-butoxide and after 24 hours only mixed fractions of the C-2 and C-4 adducts (tlc) were obtained after chromatography. The reaction was carried out again with sodium hydride as the base and this time chromatography isolated the 2-cyanomethylceph-3-em in 47% and the 4-cyanomethylceph-2-em in 34% yield. Spectroscopic and analytical data were identical to previous products.

Tabulating the results shows a trend in the way reaction proceeds:-

Alkylating Reagent	Percentage Yields	
	(459)	**(460)**
chloroacetonitrile	21	0
bromoacetonitrile	31	8
iodoacetonitrile	34	47

Chloroacetonitrile formed the C-4 adduct exclusively and not the predicted C-2. Bromoacetonitrile gave the C-4 as the major product and iodoacetonitrile gave the C-2 adduct as the major product. Thus from chloro- to iodoacetonitrile the tendency is for alkylation to go from C-4 to C-2 and hence indicates C-4 is harder than C-2. The conclusions from these experiments are not in agreement with previous unpublished results[181].

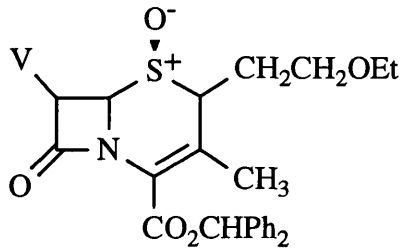
2.2.5 Attempted Alkylations of Ceph-3-ems

Benzyl Chloride

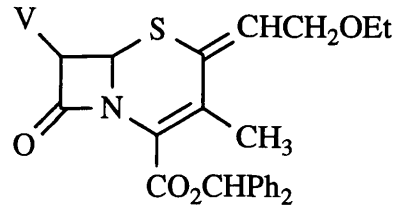
Reaction of benzyl bromide with sulphoxide **(202)** resulted in an inseparable mixture of two components of which a small amount of one product was isolated and identified as the di-adduct **(431)**. It was anticipated that benzyl chloride might have a tendency to form only one of the two products. However after 5 hours of similiar reaction conditions, the starting material remained unreacted, and after 15 hours the reaction mixture became too complicated for separation by column chromatography and was therefore no longer investigated.

Bromoethyl Ethyl Ether

Numerous attempts were made at reacting the sulphoxide **(202)** with the electrophile bromoethyl ethyl ether (a reagent used to introduce the ethoxy ethyl group into nucleophilic sites)[186]. The theoretical C-2 adduct **(461)** would be ideal for the major aim of this project ie to produce a C-2 exocyclic ceph-3-em with a good leaving group. Pummerer reaction of **(461)** according to the procedure described by Kim *et al* (section 2.3.1) would afford **(462)**.



(461)

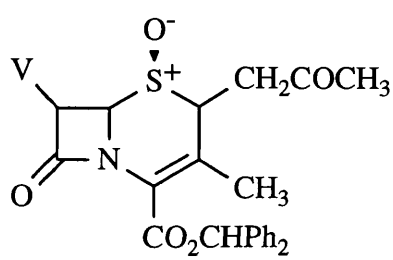


(462)

Nevertheless no reaction occurred when the sulphoxide **(202)** was stirred in the presence of triethylamine in DMF. Modifying the base to sodium hydride produced a reaction mixture that was mostly baseline material on tlc. Using potassium *t*-butoxide as base, neither starting material nor any products could be observed on tlc. Reverting back to sodium hydride the reaction was carried out at room temperature after initial addition at ice temperature. According to tlc analysis no reaction had taken place within the first two hours, but after overnight stirring, tlc indicated numerous products which were inseparable by chromatographic techniques and as a consequence, reaction of the bromoethyl ethyl ether with **(202)** was not researched further.

Chloroacetone

Reaction between chloroacetone and the diphenylmethyl ester **(202)** was also unsuccessful although there was the hope that the C-2 alkylated ceph-3-em **(463)** would be produced. Using DMF as a reaction medium, **(202)** and



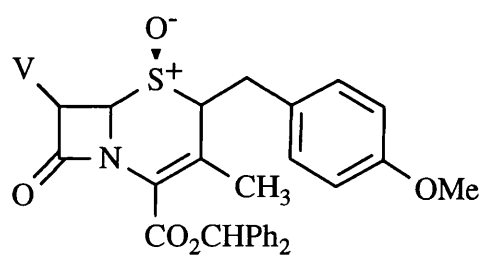
(463)

chloroacetone were stirred in the presence of sodium hydride for 24 hours after which time tlc indicated no reaction had occurred and **(202)** was still intact. A further 2.8 mmol of sodium hydride and 2 mmol of chloroacetone were added and stirring continued for another 24 hours. Again the sulphoxide **(202)** remained unreacted.

Modifying the conditions by reducing the volume of DMF; using a large excess of chloroacetone and introducing potassium iodide in an attempt to assist reaction also failed and the reaction was not investigated further.

p-Methoxybenzyl Chloride and *p*-Nitrobenzyl Chloride

P-Methoxybenzyl chloride and *p*-nitrobenzyl chloride were selected for reaction with **(202)** because of the activating and deactivating properties of the substituents attached to the benzene ring. The methoxy group donates electrons to the aromatic ring, creating a molecule less susceptible to nucleophilic attack. Hence, if reaction were to occur, few products would result giving rise to easier chromatography and a higher yield of the desired C-2 product **(464)**. In practice



(464)

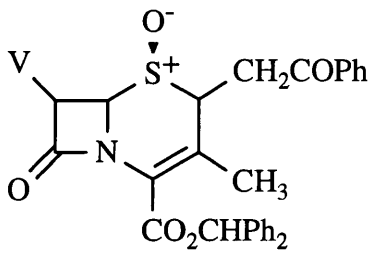
however the *p*-methoxybenzy chloride remained completely unreactive to the ceph-3-em nucleophile when sodium hydride base was employed at 0°C under a blanket of nitrogen and thus the reaction was not investigated further.

On the other hand the electrophile *p*-nitrobenzyl chloride is more susceptible to nucleophilic attack due to the nitro group generating an electron deficient ring as was evident in its reaction with the sulphoxide **(202)**, ie numerous

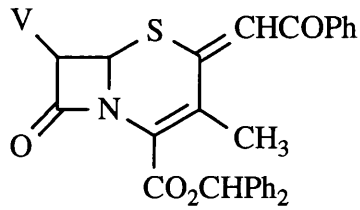
products were observed on tlc of which only starting material **(202)** was isolated by chromatography.

Phenacyl Bromide

Theoretically reaction of the ceph-3-em nucleophile with phenacyl bromide would produce the 2-adduct **(465)** which with the presence of the carbonyl function furnishes more acidic C-2 methyl hydrogens. Thus, Pummerer rearrangement of **(465)** should hypothetically proceed with a good yield of **(466)**.



(465)



(466)

Unfortunately directly adding phenacyl bromide to the sulphoxide in DMF in the presence of sodium hydride afforded an oil containing products of degradation, according to ir spectroscopy which gave no indication of the presence of β-lactam containing components. Adding phenacyl bromide dropwise over a 30 minute period gave a complex reaction mixture, inseparable by column chromatography but ir indicated the β-lactam ring was intact. Performing the reaction again under the same conditions, ie adding the phenacyl bromide dropwise but over 2 hours, gave an oil from which the starting material **(202)** was isolated. According to ir the residual oil contained products that did not exhibit the β-lactam ring carbonyl on ir, thus the signal for this carbonyl in the previous reaction mixture must have been the result of unreacted starting material.

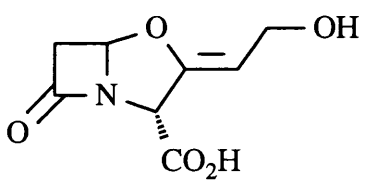
Phenacyl Chloride

Changing to phenacyl chloride resulted in a longer reaction time (24 hours) and again only products due to the degradation of the β-lactam ring were present in the reaction mixture according to ir. In conclusion, phenacyl bromide and phenacyl chloride resulted in cleavage of the vital β-lactam ring and were therefore not researched further.

2.3 Reactions at C-2 to Form Exocyclic Double Bonds

The formation of double bonds exocyclic to the dihydrothiazine ring at the 2-position of ceph-3-ems, has already been detailed in Section 1.3.3.

Clavulanic acid (**467**), a penicillin-like molecule, was isolated in 1976 from *Streptomyces clavuligerus* by Howarth and Brown[187]. It displays strong irreversible inhibition properties against β-lactamase enzymes and has the ability to protect any β-lactam antibiotic from the detrimental effects of β-lactamases. Therefore, application of penicillins along with clavulanic acid, show an significant increase in activity. Commercially, clavulanic acid is available with amoxicillin as Augmentin.

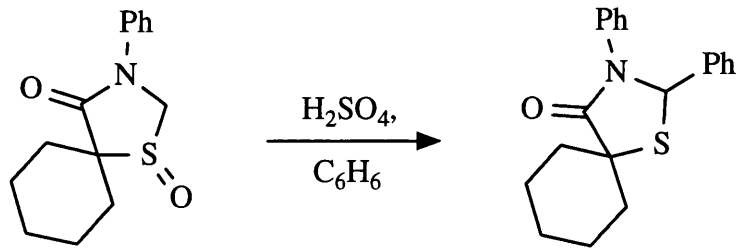


(467)

In this work, it was anticipated that by emulating the clavulanic acid molecule, a ceph-3-em might display similiar β-lactamase inhibiting properties. It was also speculated that a ceph-3-em, incorporating a 2-exocyclic double bond with either a good leaving group or an electronegative group, might display anti-bacterial activity.

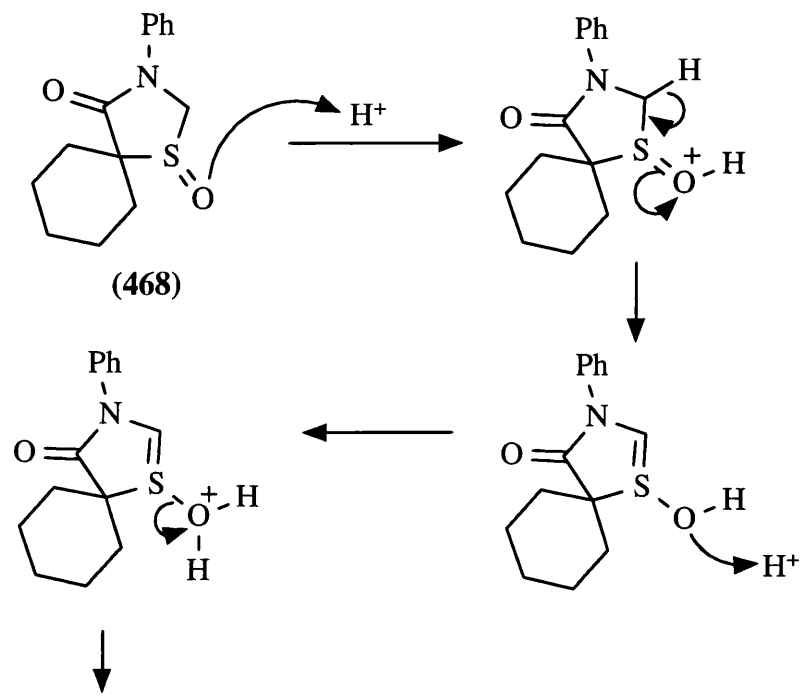
2.3.1 Pummerer Rearrangements

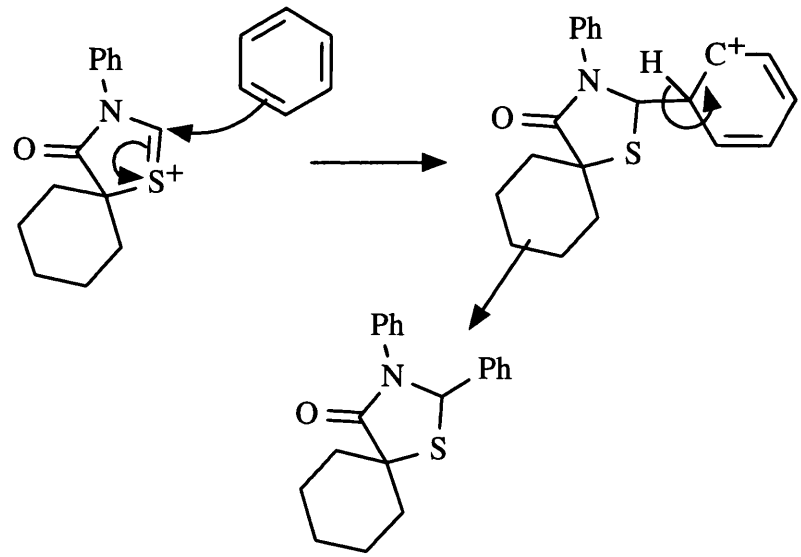
The Pummerer reaction involves[188] the functionalisation of the α-position to a sulphoxide to produce an α-substituted sulphide eg concentrated sulphuric acid in benzene promotes phenylation of **(468)** to give **(469)** as shown in Scheme 24.



(468) **(469)**

Mechanistically (Scheme 25) the Pummerer rearrangement depends on an electrophile which activates the sulphoxide and converts the oxygen into a good leaving group. Usually a base is present to remove the proton, but in the presence of sulphuric acid a proton is lost automatically and a base is not required. The final step is the incorporation of a nucleophile to afford the final product.





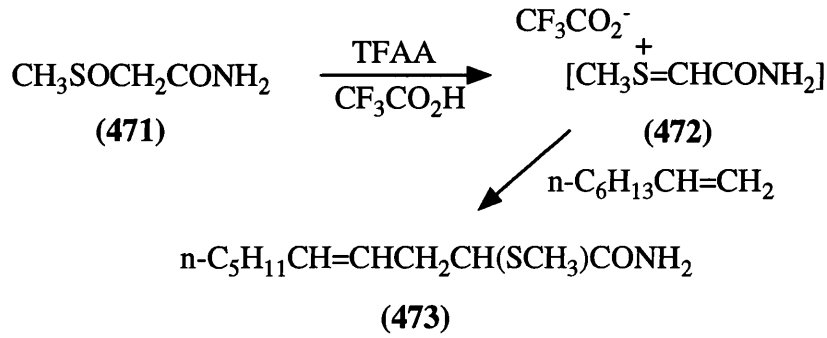
(469)

Mineral acids were the first reagents used by Pummerer. His first publication[189] on this type of reaction described heating phenylsulphinylacetic acid **(470)** with either sulphuric or hydrochloric acid to afford thiophenol and glyoxylic acid.

$$\underset{\textbf{(470)}}{PhSOCH_2CO_2H} \xrightarrow{HCl/H_2SO_4} PhSH + HCOCO_2H$$

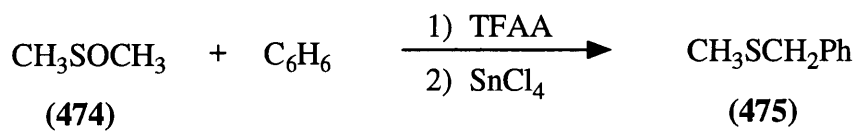
The most frequently utilised reagent is acetic anhydride which is used in large excess as the solvent or in solvents. Trifluoroacetic anhydride is often suggested as it increases the yield of products by reducing the number of side reactions that can take place[190]. In addition it promotes the Pummerer reaction under mild conditions.

Trifluoroacetic anhydride in conjunction with trifluoroacetic acid induces[191] the Pummerer intermediate **(472)**, from α-methylsulphinylacetamide **(471)**, which can then react with an alkene to produce **(473)** as shown in Scheme 26.

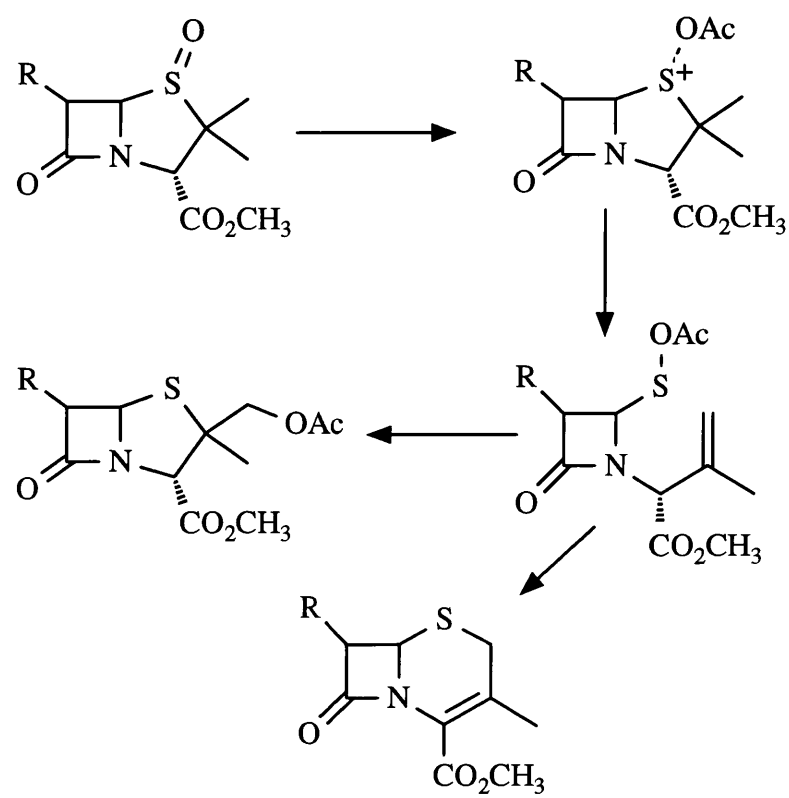


Scheme 26

A mixture of trifluoroacetic anhydride with tin tetrachloride generates benzylic thioethers (**475**) from unactivated substrates such as (**474**)[192].

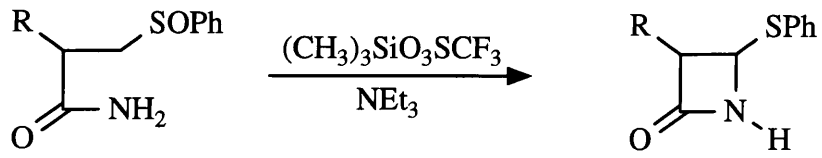


The Pummerer reaction has been widely used[19&140] in the conversion of penam systems into ceph-3-ems as shown in Scheme 27.



Scheme 27

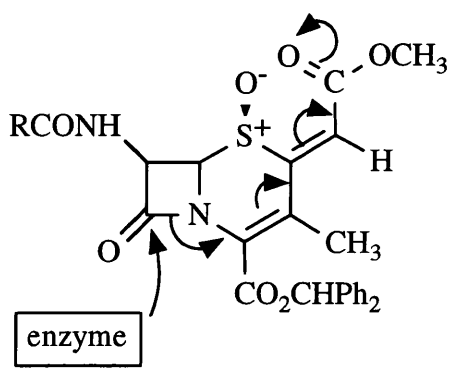
In addition[193] it has been used to convert the sulphoxide **(476)** into β-lactam **(477)** in the presence of trimethylsilyl trifluoromethanesulphonate within 15 minutes at -20°C.



(476) **(477)**

Not only can the α-sulphur substituted carbocations be trapped by nucleophiles but they can also lose a proton to give the vinyl sulphide as shown by Kim, Misco, Haynes and Macgregor[194].

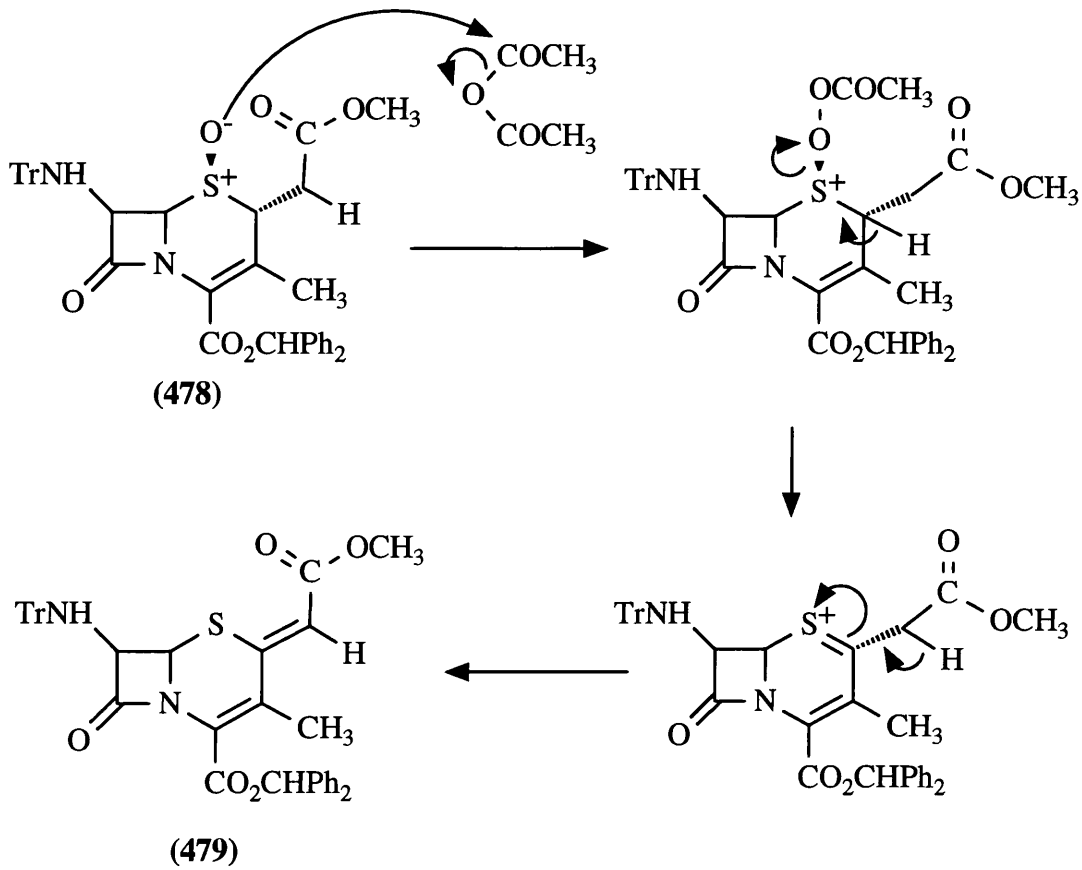
Kim *et al*[194] investigated the incorporation of the methoxy carbonyl group on the C-2 ethylidene group, in the hope that it would increase the reactivity of the β-lactam carbonyl and hence intensify the biological activity by conjugation with the Δ3-double bond as shown in Scheme 28.



Scheme 28

Thus they reacted ceph-3-em **(478)** with methyl bromoacetate in the presence of LDA at -25°C and produced the 2α-alkylated ceph-3-em **(478)** which when exposed to trifluoroacetic anhydride, acetic anhydride and 2,6-lutidine was transformed into 2-(2'-methoxycarbonyl)methylene ceph-3-em **(479)** via a Pummerer rearrangement. The proposed reaction pathway is shown in Scheme 29 whereby acetic anhydride is used as an activating electrophile. Thus, the

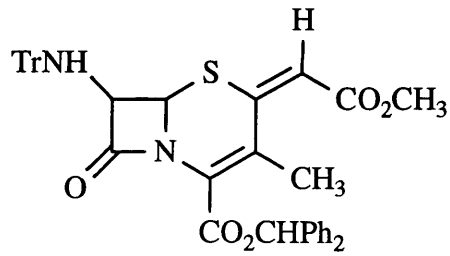
sulphoxide is activated by attacking the carbonyl of the acetic anhydride molecule which results in the C-2 proton being removed by the base present and loss of an acetic acid molecule. The more thermodynamically stable isomer is formed by migration of the double bond.



Tr = Ph_3C

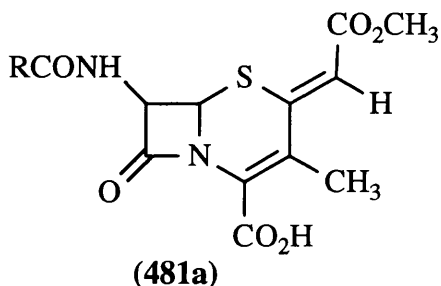
Scheme 29

Nuclear Overhauser effects were applied in order to ascertain the configuration of **(479)**. Irradiating the 3-methyl group increased the signal of the vinyl proton by 30% - strongly indicating the Z-arrangement. Ceph-3-em **(479)** was isomerised photochemically to a 35:65 mixture of **(479)** and its isomer **(480)** which was crystallised out in a 43% yield. The stereochemical arrangement of **(480)** was corroborated when no increase in the signal for the C-2 vinyl proton was observed using NOE technique.

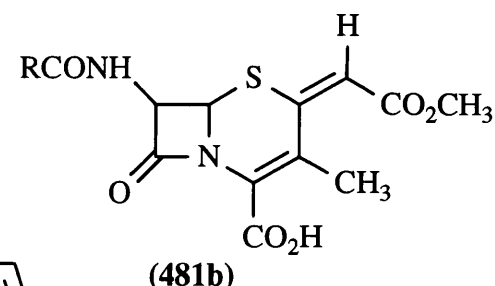


(480)

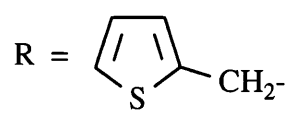
Reaction of **(479)** with 2-thienylacetylchloride in the presence of triethylamine followed by de-esterification in the usual manner afforded **(481a)** which displayed good overall antibacterial activity compared to its trans isomer **(481b)**.



(481a)

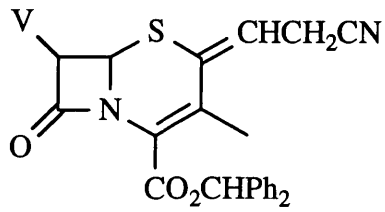


(481b)



In this work the 2-cyanoethylceph-3-em **(368)** was reacted according to the literature method, ie stirring **(368)** 2,6-lutidine, trifluoroacetic anhydride and acetic anhydride under a nitrogen atmosphere at 0°C. One major product was isolated which displayed both the nitrile and β-lactam carbonyl signals on ir (2250 and 1782 cm^{-1} respectively). According to nmr, the protons for the 3-CH_3, the phenoxyacetamido side chain, 6-H, 7-H, and the diphenylmethyl ester, were in agreement with the ceph-3-em nucleus. A further two protons were observed at δ3.29 and appeared as a double double doublet with coupling constants 6.3, 7.5 and 19 Hz. The chemical shift of δ3.29 corresponds to a CH_2 surrounded by an unsaturated system and incorporating a nitrile group. Furthermore a double doublet with chemical shift of δ6.19 and coupling constants 6.3 and 7.5 Hz was assigned to the 2-methylene proton, which coupled with the adjacent CH_2 protons

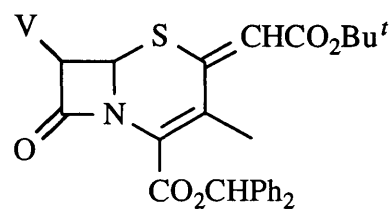
and explains the complexity of the signals. Thus, the major product had successfully undergone a Pummerer type reaction and was assigned the structure **(482)** which was afforded in 40%.



(482)

Confirmation of this structure was supplied by both mass spectroscopy (583 MNH_4^+ and 566 MH^+) and elemental analysis figures.

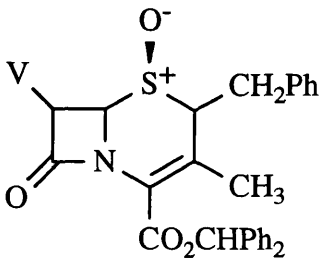
Under similiar conditions, 2-(2'-*t*-butyloxycarbonylmethyl) ceph-3-em **(436)** was rearranged in a Pummerer-type reaction to give one major product which showed the β-lactam carbonyl signal on ir. From nmr a singlet was observed with a chemical shift of δ1.51 integrating for nine protons indicative of the *t*-butyl group on C-2 and a singlet at δ6.26 integrating for one proton gives evidence for a hydrogen involved in an unsaturated system. The remainder of the spectrum was in accord with the phenoxyacetamidoceph-3-em structure containing a diphenylmethyl ester. Mass spectroscopy of this major product indicated a molecular weight of 626 which corresponds to $C_{35}H_{34}N_2O_7S$ and as a result the product was identified as **(483)**.



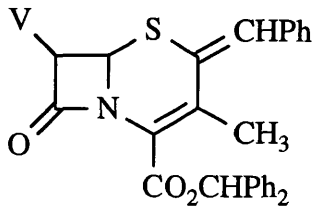
(483)

A Pummerer rearrangement of the 2-benzylceph-3-em **(432)** would furnish **(484)** - the corresponding sulphide of the product from reaction of benzaldehyde and the sulphoxide **(202)** had they reacted in a Knoevenagel

condensation (see section 2.3.3). Unfortunately **(432)** was not isolated from the

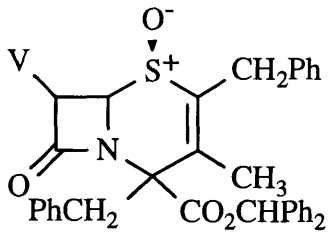


(432)



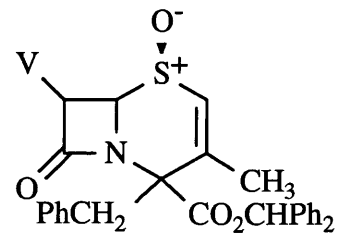
(484)

alkylation of **(202)** with benzyl bromide. However, since previous alkylation reactions gave inseparable mixtures of di-adducts and C-2 substituted mono-adducts, it was surmised that the 2,4-dibenzyl di-adduct **(431)** was contaminated with the desired ceph-3-em **(432)**. Therefore a Pummerer-type



(431)

reaction was attempted on the di-adduct mixture in the hope that **(431)** would remain unreacted and the product **(484)** from suspected **(432)** could be isolated. After 30 hours of stirring and a further addition of 2,6-lutidine two compounds were isolated. The major of the two existed as a ceph-2-em as indicated by vinylic coupling between the 2-H and 3-CH_3. These signals appeared at δ6.09 and δ1.78 respectively as doublets with a coupling frequency of 0.84 Hz. An AB quartet at 3.35 and 3.92 was assigned to the CH_2 from the benzyl group which had to be situated at the C-4 position in order to accomodate the Δ2 double bond. Furthermore the usual multiplet at δ7.0 integrated for an extra 5 protons giving evidence for the phenyl ring from the benzyl group, hence the major component was assigned the structure **(485)**.



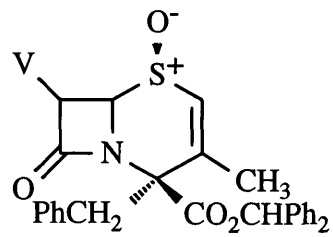
(485)

Mass spectroscopy displayed two molecular ions ie 621 and 638 which support structure **(485)** with a molecular formula of $C_{36}H_{32}N_2O_6S$ and a molecular weight of 620. Thus 621 represents MH^+ and 638 MNH_4^+ and in microanalysis figures also correspond to and reinforce structure **(485)**.

4-Benzyl ceph-2-em **(485)** was not a product from this reaction and was therefore present in the original mixture. It has since become separable, as the di-adduct **(431)** appears to have degraded under these conditions.

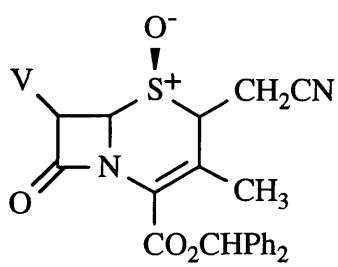
The second component isolated exhibited a similiar nmr spectrum to ceph-2-em **(485)**. The only differences were the CH_2 from the phenoxyacetamido side chain and the 6-H emerged as two separate signals, as opposed to the multiplet for **(485)**, and the 2-H and the 3-CH_3 with chemical shifts at 6.54 and 1.61 appeared as singlets, apparently not coupling. The 2-H however must be part of an unsaturated system for the signal to appear so far downfield. In addition, microanalysis and mass spectroscopy support the formula $C_{36}H_{32}N_2O_6S$ with molecular weight 620. Again from the analytical and spectroscopic data the 4-benzyl structure is speculated and therefore the second component is an isomer of **(485)** and likewise must have been present in the original mixture and not a product from this reaction. Ceph-2-em **(485)** is the major isomer where alkylation has occured on the β-face. It has been postulated[37] that alkylation at C-4 is hindered on the α-face because the carbanion is forced onto the β-face as a result of the repulsion of the N-5 lone pair (on the α-face). Thus one isomer exists for

the second component, ie for alkylation to occur on the hindered α-face and hence, resulting in the ceph-2-em (**486**). However this should have no effect on the vinylic coupling between the 2-H and 3-CH_3 and so the structure remains tentatively assigned.

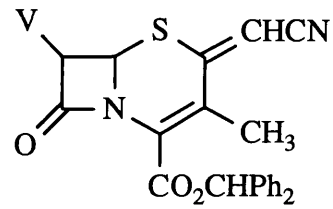


(**486**)

The 2-cyanomethyl ceph-3-em (**460**), having a similiar structure to the 2-cyanoethyl structure (**368**) but containing a more acidic hydrogen was expected to give a good yield of the 2-cyanomethylene product (**487**) under Pummerer rearrangement conditions. However, after 15 hours, a complex mixture of compounds was obtained according to tlc, and an attempt at separation by column chromatography failed. In contrast to the 2-cyanoethyl adduct (**368**), (**460**) had



(**460**)

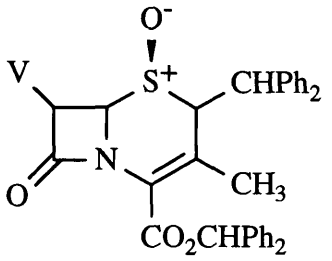


(**487**)

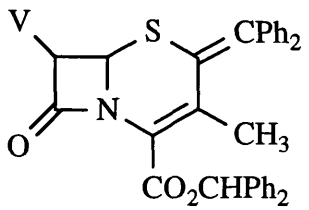
enhanced α-hydrogen acidity as a result of the adjacent strong electron withdrawing group. Hence it is speculated that its increased reactivity has induced other reactions to take place resulting in a complex reaction mixture as observed on tlc..

Likewise the 2-diphenylmethyl ceph-3-em (**435**) incorporates a more acidic α-hydrogen and was therefore also expected to react efficiently to give (**488**)

from this rearrangement. However it was discovered that **(435)** reacted only after 48 hours, required the addition of two 0.15 ml quantities of 2,6-lutidine and gave an inseparable mixture of products. It is also possible that the quantity of 2,6-lutidine added resulted in various reactions occurring and the degradation of the β-lactam ring.



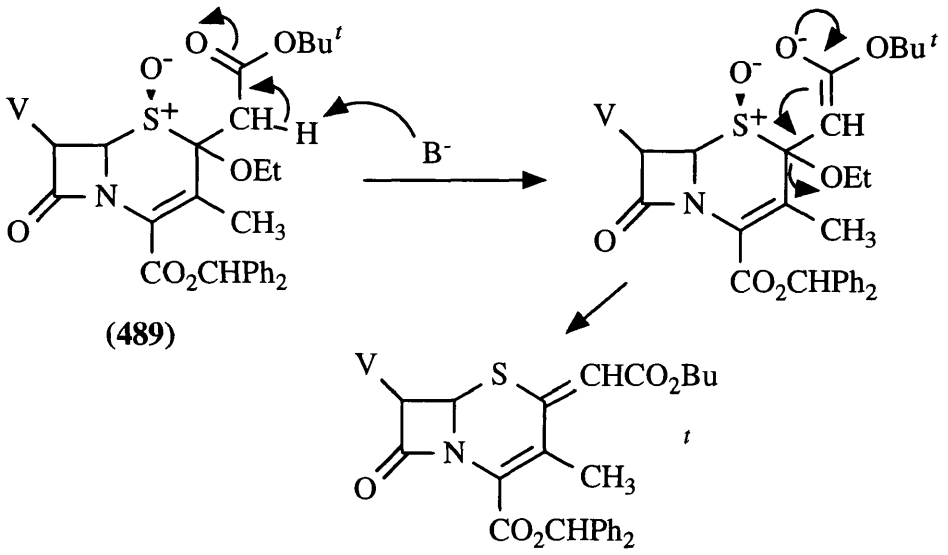
(435)



(488)

2.3.2 Attempted Elimination of C-2 Ethoxy Group

Hypothetically (Scheme 30) adding a base to a solution of **(489)** would initiate the removal of an acidic α-hydrogen to the *t*-butyl ester group and, consequently, rearrangement would culminate in the loss of the ethoxy group to provide a ceph-3-em **(483)** with the desired C-2 exocyclic double bond.

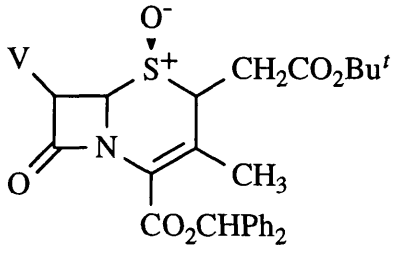


(489)

(483)

Scheme 30

Thus in order to prepare (**489**), the sulphoxide (**436**) was dissolved in dry THF and stirred at room temperature overnight with ethyl chloroformate.

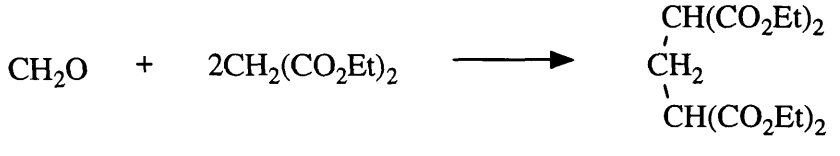


(**436**)

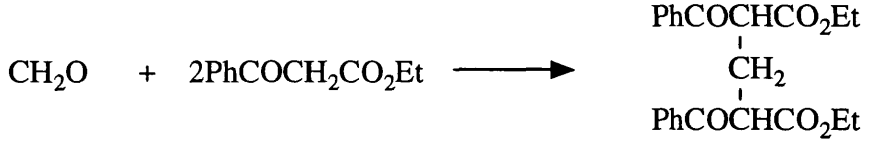
According to tlc, three products were present (after chromatography removed the unwanted baseline material) with almost the same Rf's and attempts to separate further these compounds failed. Assuming one of the three might be the desired compound (**489**), the mixture was stirred in dry THF with triethylamine in the hope that one of the three products would undergo the elimination. After four hours an even more complex mixture of compounds were observed by tlc. An attempt at chromatography failed and the reaction was not investigated further.

2.3.3 Attempted Knoevenagel Reaction with Benzaldehyde

The first publication produced by Knoevenagel[195] involved the condensation of formaldehyde with diethyl malonate and ethyl benzoylacetate, using ethylamine as a base, to afford the bis products (**490**) and (**491**) respectively.

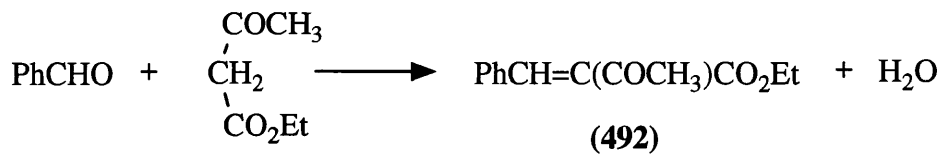


(**490**)



(**491**)

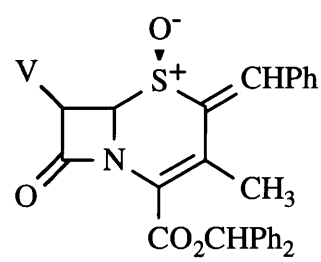
A later report[196] described the condensation of benzaldehyde with ethyl acetoacetate in the presence of piperidine at ice temperature to produce ethyl benzylidene acetoacetate **(492)**.



(492)

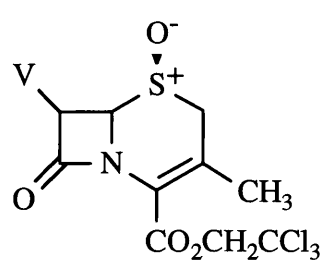
The Knoevenagel reaction is applicable to any aldehyde condensing with any active methylene group, although conditions vary drastically depending on the reactivity of the methylene group.

In the presence of base, both hydrogens of C-2 have been removed and consequently it was believed that **(202)** would undergo a base catalysed Knoevenagel-type reaction with benzaldehyde to give the 2-exomethylene compound **(493)**.



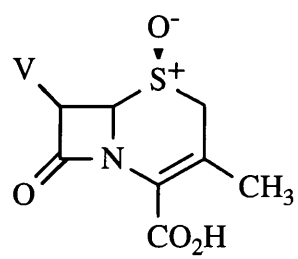
(493)

Previous attempts[181] using the trichloroethyl ester **(51)** and triethylamine base in both toluene and acetonitrile solvents at various temperatures proved unsuccessful.



(51)

Modifying the ester to diphenylmethyl ester had no apparent effect. Refluxing **(202)** in freshly distilled benzaldehyde resulted in degradation of the β-lactam ring, as indicated by the complete lack of the β-lactam carbonyl stretching frequency on ir. Another attempt involved refluxing **(202)** in benzaldehyde at 100°C in the presence of triethylamine and zinc chloride for 1 hour, after which time tlc indicated all starting material had reacted and the presence of the β-lactam carbonyl stretch was detected by ir (1793 cm^{-1}). From nmr, the most noticeable difference involved the multiplet at δ7 for the diphenylmethyl ester which normally integrated for approximately 16 protons. In this case a multiplet was observed at δ6.81-7.29 which only integrated for 5 protons corresponding to the phenyl group from the phenoxyacetamido side chain and thus removal of the diphenylmethyl ester group had occurred. The presence of a proton upfield from δ10.0 was indicative of an acid group and the remainder of the spectrum corresponded to the ceph-3-em **(202)**. Hence the only reaction which has occurred is de-esterification and therefore the corresponding acid structure **(494)** was assigned.

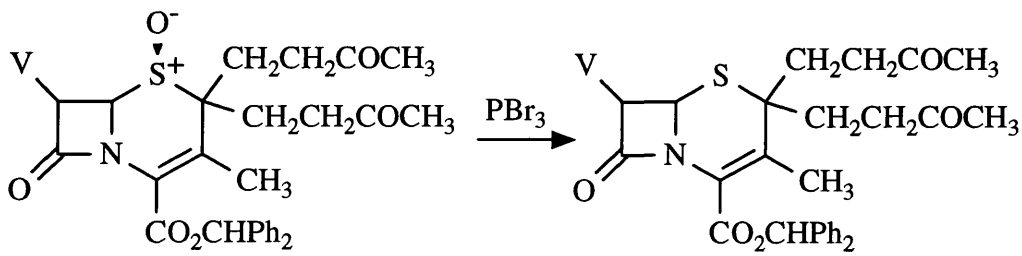


(494)

The reaction conditions were slightly modified and acetic anhydride was utilised in place of zinc chloride. According to tlc the starting material **(202)** was consumed within 1.5 hours and although β-lactam containing products were present (ir), they were too numerous for a successful separation. As a result of these poor results and previous fruitless research the reaction was not investigated further.

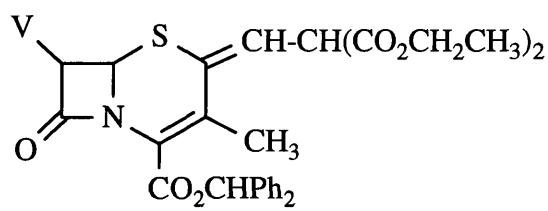
2.4 De-oxygenation

Initially de-oxygenation of the di-adduct (383) was attempted using a previous published method[22]. After 24 hours of stirring with acetyl chloride and potassium iodide, the starting material was recovered unreacted. However using PBr_3[22], a solution of the di-adduct in DMF was de-oxygenated within 35 minutes to give one major product contaminated with two minor components as indicated by tlc. Purification by chloroform chromatography was attempted to remove traces of petrol and ethyl acetate with no reasonable results and hence microanalysis figures were not helpful in determining the structure. From nmr spectroscopy two methyl groups appeared as singlets at δ2.12 and δ2.14 signifying the $COCH_3$ groups similiar to the di-adduct sulphoxide **(383)** but the multiplet for CH_2CH_2CO has shifted upfield to δ2.39-2.96 as would be expected for the corresponding sulphide of **(383)**. Additionally the H-7 and N-H have moved upfield and the H-6 has moved downfield (as would be expected[174]) compared to the sulphoxide. The remainder of the spectrum is similiar to the sulphoxide. The mass spectrum indicates a molecular ion of 655 (MH^+), a difference of 16 amu from the sulphoxide (671 amu) which verifies the loss of oxygen. As a result the major product was deduced as having the structure **(495)** which was produced in a 61% yield.



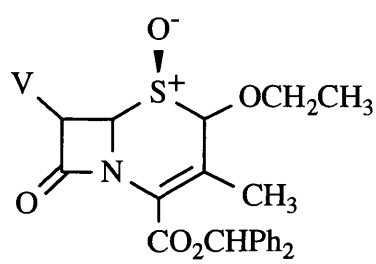
(383) **(495)**

De-oxygenation of the 2-diethylmethylene malonate adduct (390) was accomplished at ice temperature in the presence of acetyl chloride and potassium iodide. One major product which displayed the β-lactam carbonyl signal (1785 cm^{-1}) was isolated from chromatography. According to nmr the chemical shifts for 2-CH, 7-H and N-H have all moved upfield and the 6-H signal has moved downfield compared to the signals for the corresponding sulphoxide, as would be expected for the sulphide. The remainder of the spectrum was similiar to that of (390). Furthermore both microanalysis figures and mass spectroscopy indicated that the molecular formula was $C_{37}H_{36}N_2O_9S$ and as a result it was concluded that de-oxygenation had taken place and structure **(496)** was assigned for the major product.



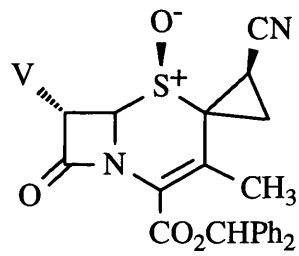
(496)

An attempt was also made at de-oxygenating the 2-ethoxy ceph-3-em minor product **(392)** from Michael addition of **(202)** and diethyl ethoxymethylenemalonate. Using the same conditions ie acetyl chloride and potassium iodide at ice temperature, a complex mixture of products were obtained according to tlc. Attempts at successful column chromatography failed and therefore the reaction was abandoned.

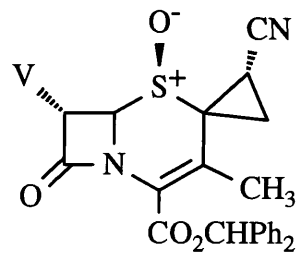


(392)

In an effort to verify the speculated structure of 2-(2'-cyanocyclopropyl) ceph-3-em from reaction of 2-chloroacrylonitrile and **(202)**, the major two products were de-oxygenated.

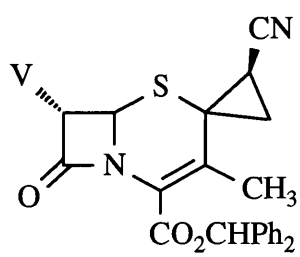


(382a)

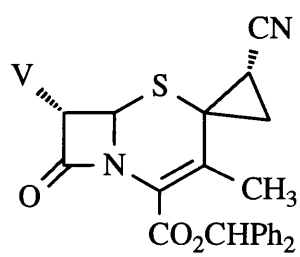


(382b)

Sulphoxide **(382a)** was de-oxygenated in the presence of phosphorus tribromide at 0°C in DMF. Column chromatography isolated one product which displayed both the nitrile and the β-lactam carbonyl stretching frequencies on ir (2240 & 1788 cm^{-1}). Mass spectroscopy gave a molecular ion of 583 (MNH_4^+) indicating the loss of an oxygen (599 MNH_4^+ for sulphoxide **(382a)**). Three protons were observed on nmr as a similiar multiplet as the sulphoxide. Therefore it is concluded that the de-oxygenated product is an isomer **(497a)** which was afforded in 15% yield. Structure **(497a)** was further supported by microanalysis figures, ie the found percentage values of the elements were C,67.87; H,5.01; N,7.04 and S,5.41 and are similiar to the required percentage values for $C_{32}H_{27}N_3O_5S$.



(497a)



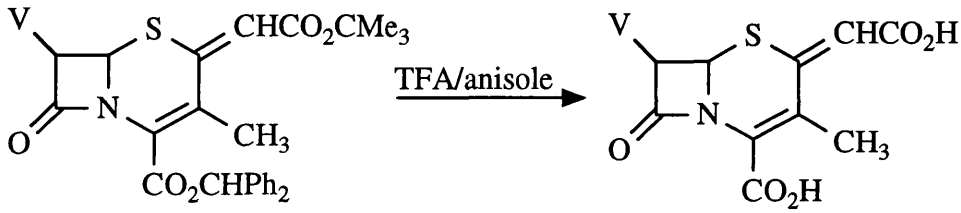
(497b)

Likewise **(382b)** was de-oxygenated using phosphorus tribromide and ir of isolated product indicated the presence of both nitrile and β-lactam functions, 2238 and 1777 cm^{-1} respectively. Again mass spectroscopy gave a molecular ion of 583 for MNH_4^+ - a decrease of 16 amu compared to the corresponding sulphoxide and hence indicating the loss of oxygen.

In addition the microanalysis figures are in agreement with those required for the structure **(497b)** with formula $C_{32}H_{27}N_3O_5S$.

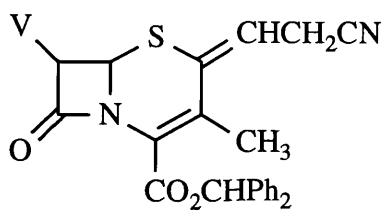
2.5 De-esterification

De-esterification of the 2-*t*-butyloxycarbonylmethylene adduct **(483)** would afford the di-acid **(498)**. However, although numerous attempts were undertaken using anisole and trifluoroacetic acid[197], the end result was always a product that did not contain the β-lactam carbonyl stretching frequency according to ir spectroscopy.

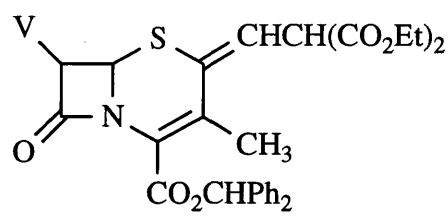


(483) **(498)**

Likewise the 2-cyanoethylene ceph-3-em **(482)** and 2-diethyl methylene malonate ceph-3-em **(496)** decomposed on attempted de-esterifications with anisole and trifluoroacetic acid.



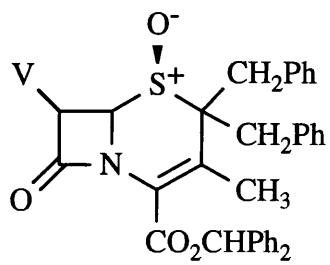
(482)



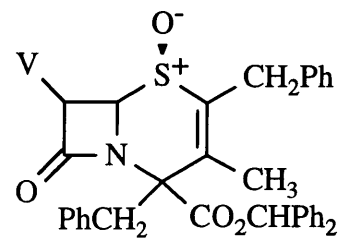
(496)

2.6 Determination of Di-adduct Configuration

The benzyl di-adduct (from reaction of sulphoxide **(202)** with benzyl bromide) has a possibility of two structures ie 2,2-dibenzyl ceph-3-em **(430)** or the 2,4-dibenzyl ceph-2-em **(431)**. Both of these structures would have similiar nmr, and so by the spectroscopic or analytical techniques applied, there was no positive identification of which isomer was the product of this reaction.

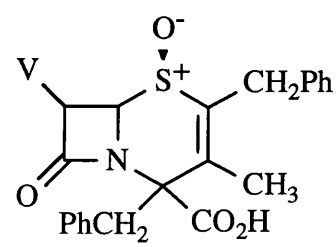


(430)



(431)

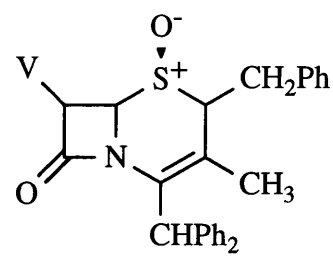
Previously C-4 substituted ceph-2-ems were reported[88] to de-esterify and further decarboxylate, if the prepared acid **(499)** was stirred overnight in the presence of triethylamine. Hence a possible way of determining which of the di-adduct isomers exist would be to attempt a decarboxylation. Thus isomer **(431)** would undergo decarboxylation and isomer **(430)** would not.



(499)

Utilising trifluoroacetic acid and anisole in a dichloromethane medium, the di-adduct was de-esterified after 1 hour as indicated by tlc. Dichloromethane was replaced by ethyl acetate and an excess of triethylamine was added. After stirring overnight, work up followed by chromatography afforded an oil that

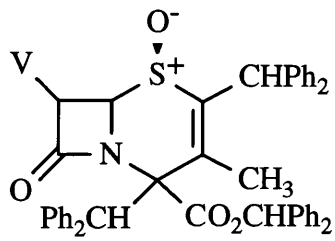
displayed β-lactam properties (ir 1783 cm^{-1}). From nmr spectroscopy, a multiplet for 2 hydrogens between δ2.77 and δ3.12 represents the 2-CH_2Ph and two doublets both integrating for one proton at δ3.97 and δ4.26 with coupling constants of 15.5 Hz correspond to the 4-CH_2Ph which is adjacent to the Δ3-double bond. Additionally, a multiplet at δ3.65-3.67 integrating for 1 proton signifies the 2-H. The principal difference is the absence of the diphenylmethyl ester group. A multiplet from δ6.88-7.33 integrating for 15 protons represents the phenyl group from the phenoxyacetamido side chain and the aromatic rings from the benzyl groups. In addition no acid group was observed on either nmr or ir, indicating decarboxylation had in fact taken place and as a result the 2,4-dibenzyl ceph-2-em isomer **(500)** was assigned to this compound.



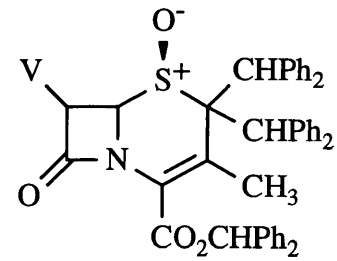
(500)

Both mass spectroscopy, which gave a molecular ion of 500 amu, and elemental analysis figures which corresponded to the formula $C_{29}H_{28}N_2O_4S$, contributed evidence to the proposed structure of **(500)**. Consequently, the original starting material had to have been the 2,4-dibenzyl ceph-2-em **(431)**.

A similiar problem existed with the di-adduct from reaction of **(202)** with bromodiphenylmethane. Again two isomers ie **(433)** and **(434)** were possible.



(433)

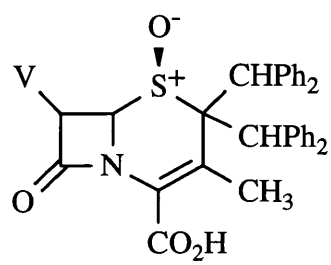


(434)

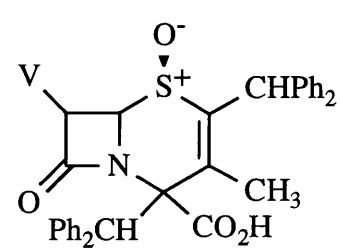
Speculation suggested the **(433)** isomer, due to steric hindrance of the bulky phenyl groups affected the incoming diphenylmethyl group from the second addition. To verify this conjecture, the de-esterification and decarboxylation procedure was attempted.

According to tlc, de-esterification proceeded within one hour using trifluoroacetic acid and anisole. The dichloromethane was replaced with ethyl acetate and stirring continued overnight at room temperature with triethylamine. After 24 hours decarboxylation had not taken place according to tlc. Attempted recovery of the suspected acid **(501)** failed and the reaction was abandoned.

An attempt at isolating the acid was unsuccessful so it is not certain whether or not the desired acid **(502)** for decarboxylation was formed, and since decarboxylation did not take place it is concluded that the original di-adduct is the 2,2-diphenylmethylceph-3-em **(434)**.



(501)



(502)

Attempts at converting the novel ceph-3-ems (prepared in this work) to their corresponding acids were unsuccessful and as a result no biological testing was carried out.

EXPERIMENTAL

3.0 EXPERIMENTAL

Infrared spectra were recorded on a Perkin-Elmer 1600 FT-IR; nuclear magnetic resonance spectra were recorded in deuterochloroform, unless otherwise specified, on a Bruker WM250 at 250 MHz or Joel JMN PMX6051 spectrometer at 60 MHz using tetramethylsilane as an internal reference and mass spectra measured with a VG 7070. Melting points were determined using an Electrothermal melting point apparatus and are uncorrected. Column chromatography was accomplished using pressurised short path columns with Kieselgel 60, particle size < 0.063mm (Merck # 7729). Reactions were monitored by thin layer chromatography on Merck DC-Alufolien Kieselgel 60 F_{254} (Merck # 5554) plates which were visualised using ultra-violet irradiation; potassium permanganate solution or iodine vapour.

Diphenylmethyl (6R,7R) 3-methyl-7β-phenoxyacetamidoceph-3-em 4-carboxylate was gifted by Glaxochem, Montrose.

Attempted Reaction of Diphenylmethyl (6R,7R) 3-Methyl-7β-phenoxyacetamido-ceph-3-em-4-carboxylate 1β-oxide (202) with Acrylonitrile using Triethylamine.

Triethylamine was added to a stirred solution of the sulphoxide **(202)** (1.0g, 2 mmol) in acrylonitrile (40 ml) at room temperature for 24 hours. Solvents were removed *in vacuo* and the residue partitioned between ethyl acetate and dil HCl. The organic phase was washed with saturated aqueous $NaHCO_3$ solution, brine, dried ($MgSO_4$) and concentrated under reduced pressure to give a complex mixture of products inseparable by column chromatography.

Synthesis of Diphenylmethyl (6R,7S) 2α-(2'-cyanoethyl)-3-Methyl-7α-phenoxyacetamidoceph-3-em-4-carboxylate 1β-oxide (369).

To a suspension of the sulphoxide **(202)** (1.0g, 2 mmol) in acrylonitrile (15 ml) at room temperature, triethylamine (2 ml) was added. The reaction mixture was gently heated until all the starting material had dissolved and then cooled to room temperature. Stirring was continued overnight and then the solvent evaporated *in vacuo*. The residue was dissolved in ethyl acetate; washed with dil HCl, brine, saturated aqueous $NaHCO_3$, brine; dried ($MgSO_4$) and evaporated *in vacuo* to give a complex mixture of products of which ***diphenylmethyl (6R,7S) 2α-(2'-cyanoethyl)-3-methyl-7α-phenoxyacetamidoceph-3-em-4-carboxylate 1β-oxide (369)*** was separated by chromatography and recrystallised from ethyl acetate/petrol to give light orange crystals (0.17g, 15%); mp 185-187°C; υ_{max} 3418, 2248, 1791, 1640 and 1698 cm^{-1}; δ (250 MHz) 1.19-1.59 (2H, m, 2-C$\underline{H}_2$CH_2CN), 1.91 (3H, s, 3-CH_3), 2.43-2.52 (2H, m, 2-CH_2C$\underline{H}_2$CN), 3.41 (1H, dd, J=3.25 & 10.1 Hz, 2-H), 4.50 (2H, s, CH_2CON), 4.56 (1H, d, J=2.25 Hz, 6-H), 5.28 (1H, dd, J=2.25 & 7.6 Hz, 7-H), 7.89-7.45 (16H, m, PhO & $CHPh_2$) and 7.67 (1H, d, J=7.6 Hz, N-H); m/e 606 MNa^+.

Tlc Analysis of Reaction of (202) with Acrylonitrile.

Triethylamine (1 ml) was added to a stirred solution of the sulphoxide **(202)** (0.5g, 1 mmol) in acrylonitrile (10 ml) at room temperature. The reaction was monitored by tlc every 10 minutes for the first hour; then every 15 minutes for the second hour and every 30 minutes for the last two hours. Within the first hour, two products were observed although the majority of starting material remained unreacted. Over the next 2 hours, four more products appeared as well as baseline material. Over the last two hours, the tlc plate became streaky with the presence of numerous products.

Reaction of Sulphoxide (202) with Acrylonitrile.

A solution of the sulphoxide (1.67g, 3.2 mmol) and triethylamine (1.5 ml) in acrylonitrile (30 ml) were heated at reflux for 2 hr. Tlc of reaction mixture indicated the presence of numerous products inseparable by chromatography.

Synthesis of Diphenylmethyl (6R,7R) 2α-(2'-Cyanoethyl)-3-Methyl-7β-phenoxyacetamidoceph-3-em-4-carboxylate 1β-oxide (368).

Triton B (5 drops) was added to a stirred suspension of the ester **(202)** (2.5g, 4.7 mmol) in acrylonitrile (25 ml) and ethanol (5 ml). Stirring was continued for 1 hr and then the solvent was reduced to half the volume by evaporating *in vacuo*. Ethyl acetate (100 ml) was added and the solution washed with dil HCl, brine, saturated aqueous $NaHCO_3$, brine, dried ($MgSO_4$) and evaporated under reduced pressure. Chromatography afforded ***diphenylmethyl (6R,7R) 2,2-di(2'-cyanoethyl)-3-methyl-7β-phenoxyacetamidoceph-3-em-4-carboxylate 1β-oxide (373)*** as white crystals (1.21g, 51%); mp 128-130°C; υ_{max} 3376, 2248, 1781, 1732 and 1696 cm^{-1}; δ (250 MHz) 2.06 (3H, s, 3-CH_3), 2.80-3.01 (8H, m, 2-CH_2CH_2CN), 4.57 (2H, s, CH_2CON), 4.68 (1H, d, J=4.8 Hz, 6-H), 6.23 (1H, dd, J=4.8 & 10.4 Hz, 7-H), 6.91-7.49 (16H, m, PhO & $CHPh_2$) and 7.84 (1H, d, J=10.4 Hz, N-H); (Found: C,66.20; H,4.79; N,8.61; S,4.69. $C_{35}H_{32}N_4O_6S$ requires C,66.02; H,5.07; N,8.80; S,5.04%); m/e 659.5 MNa^+.

The title compound, ***diphenylmethyl (6R,7R) 2α-(2'-cyanoethyl)-3-methyl-7β-phenoxyacetamidoceph-3-em-4-carboxylate 1β-oxide (368)*** was isolated as a milky white solid (0.36, 17%); υ_{max} 3392, 2250, 1795, 1730 and 1698 cm^{-1}; δ (250 MHz) 1.39-1.61 (2H, m, 2-C<u>H</u>$_2$$CH_2CN$), 2.11 (3H, s, 3-$CH_3$), 2.56-2.62 (2H, m, 2-$CH_2$C<u>H</u>$_2$CN), 3.53 (1H, dd, J=3.25 & 10.1 Hz, 2-H), 4.48 (1H, d, J=4.8 Hz, 6-H), 4.59 (2H, s, CH_2CON), 6.20 (1H, dd, J=4.8 & 10.3 Hz, 7-H), 6.88-7.49 (16H, m, PhO & $CHPh_2$) and 7.89 (1H, d, J=10.3 Hz, N-H); (Found: C,65.58; H,5.14; N,7.05; S,5.31. $C_{32}H_{29}N_3O_6S$ requires C,65.85; H,5.01; N,7.20;

S,5.49%); m/e 607.2 MNa^+.

Chromatography also recovered sulphoxide **(202)**, (0.53g) and two less polar spots as a mixture (0.44g).

Reaction of (202) with Acrylonitrile using Triton B.

The reaction of **(202)** with acrylonitrile was carried out as before but the reaction time was extended to 24 hr after which time tlc indicated the starting material had disappeared. After column chromatography, the di-adduct ***(373)*** was separated in 70% yield and the mono-adduct ***(368)*** in 6%. The same mixture of less polar products was also obtained in 0.48g.

Attempted Separation of Two Less Polar Products (from Reaction of (202) with Acrylonitrile) by Oxidation.

A solution of the two less polar spots (0.48g) in dichloromethane (15 ml) was stirred with *m*-CPBA (0.16g, 1mmol) for 90 minutes. Tlc indicated a mixture of two even more less polar spots which remained inseparable by column chromatography and therefore reaction was abandoned.

Attempted Reaction of Diphenylmethyl (6R,7R) 3-Methyl-7β-phenoxy-acetamidoceph-3-em-4-carboxylate 1β-Oxide (202) with 2-Chloroacrylonitrile using Triethylamine in THF.

Triethylamine (0.57g, 6 mmol) and 2-chloroacrylonitrile (0.5g, 6 mmol) were added to a stirred solution of the sulphoxide (1g, 2 mmol) in THF (25 ml) at 0°C. Stirring was continued at room temperature overnight after which time tlc indicated starting material remained unreacted.

Attempted Reaction of Sulphoxide (202) with 2-Chloroacrylonitrile using Sodium Hydride in DMF.

The sulphoxide **(202)** (0.5g, 1 mmol), dissolved in DMF was cooled to 0°C. 2-Chloroacrylonitrile (0.21g, 2.4 mmol) was added followed by sodium hydride (0.08g, 3.3 mmol) and stirring continued for 5 hr. Tlc indicated the reaction mixture was too complex to attempt chromatography and the reaction was abandoned.

Attempted Reaction of (202) with 2-Chloroacrylonitrile.

Triethylamine (0.3g, 3 mmol) was added to a suspension of the sulphoxide (0.5g, 1 mmol) in 2-chloroacrylonitrile (5 ml). After 5 hr ir indicated the β-lactam ring had degraded and the reaction mixture was discarded.

Preparation of Diphenylmethyl (6R,7R) 2-Cyanocyclopropyl 3-Methyl-7β-phenoxyacetamidoceph-3-em-4-carboxylate 1β-Oxide (382a-d).

A solution of the sulphoxide **(202)** (5g, 9.4 mmol) in acetonitrile (50 ml) was stirred at ice temperature with 2-chloroacrylonitrile (2.48g, 28.3 mmol) and triethylamine (2.86g, 28.3 mmol). After 18 hr stirring at room temperature the solvents were removed *in vacuo* and the residue was dissloved in ethyl acetate. The resultant solution was washed with 1M HCl, water, saturated aqueous $NaHCO_3$, brine; dried ($MgSO_4$); concentrated under reduced pressure and columned to afford *diphenylmethyl* ***(6R,7R) 2-cyanocyclopropyl-3-methyl-7β-phenoxyacetamidoceph-3-em-4-carboxylate 1β-oxide (382c)*** as a white solid (0.7g, 13%); υ_{max} 3385, 2242, 1797 and 1700 cm^{-1}; δ (250 MHz) 1.70 (3H, s, 3-CH_3), 2.18-2.34 (3H, m, 2-CH_2CHCN), 4.58 (2H, s, CH_2CON), 4.81 (1H, d, J=4.8 Hz, 6-H), 6.30 (1H, dd, J=4.8 & 10.7 Hz, 7-H), 6.90-7.44 (16H, m, PhO & $CHPh_2$) and 7.80 (1H, d, J=10.7 Hz, N-H); (Found: C,65.81; H,4.77; N,7.00; S,5.25. $C_{32}H_{27}N_3O_6S$ requires C,66.08; H,4.68; N,7.23; S,5.51%); m/e 599

MNH_4^+.

The second product ***diphenylmethyl (6R,7R) 2-cyanocyclopropyl-3-methyl-7β-phenoxyacetamidoceph-3-em-4-carboxylate 1β-oxide (382d)*** was isolated as white crystals (0.38g, 7%); mp 219-221°C; υ_{max} 3378, 2247, 1795, 1725 and 1698 cm^{-1}; δ (250 MHz) 1.69-1.76 (1H, m, 2-CH_2CHCN), 1.97 (3H, s, 3-CH_3), 2.04-2.39 (2H, m, 2-CH_2CHCN), 4.55 (2H, s, CH_2CON), 4.77 (1H, d, J=4.7 Hz, 6-H), 6.17 (1H, dd, J=4.7 & 10.1 Hz, 7-H), 6.88-7.46 (16H, m, PhO & $CHPh_2$) and 7.75 (1H, d, J=10.1 Hz, N-H); (Found: C,65.88; H,4.75; N,7.11; S,5.27. $C_{32}H_{27}N_3O_6S$ requires C,66.08; H,4.68; N,7.23; S,5.51%); m/e 599 MNH_4^+ and 433 $[MNH_4\text{-}Ph_2CH+H]^+$

Also isolated was ***diphenylmethyl (6R,7R) 2-cyanocyclopropyl-3-methyl-7α-phenoxyacetamidoceph-3-em-4-carboxylate 1β-oxide (382a)*** as a white foam (1.35g, 25%); υ_{max} 3380, 2241, 1791, 1743 and 1663 cm^{-1}; δ (250 MHz) 1.52 (3H, s, 3-CH_3), 1.58-2.33 (3H, m, 2-CH_2CHCN), 4.59 (2H, s, CH_2CON), 5.06 (1H, d, J=2.3 Hz, 6-H), 5.20 (1H, dd, J=2.3 & 7.6 Hz, 7-H) and 6.93-7.47 (17H, m, PhO $CHPh_2$ & N-H); (Found; C,65.89; H,4.67; N,7.21; S,5.40. $C_{32}H_{27}N_3O_6S$ requires C,66.08; H,4.68; N,7.23; S,5.51%); m/e 599 MNH_4^+ and 433 $[MNH_4\text{-}Ph_2CH+H]^+$.

The most polar product was ***diphenylmethyl (6R,7R) 2-cyanocyclopropyl-3-methyl-7α-phenoxyacetamidoceph-3-em-4-carboxylate 1β-oxide (382b)*** obtained as a light brown foam (0.99g, 18%); υ_{max} 3340, 2245, 1789 and 1731 cm^{-1}; δ (250 MHz) 1.74 (3H, s, 3-CH_3), 1.77-1.80 (1H, m, 2-CH_2CHCN), 2.15-2.26 (2H, m, 2-CH_2CHCN), 4.53 (2H, s, CH_2CON), 4.73 (1H, d, J=2.1 Hz, 6-H), 5.39 (1H, dd, J=2.1 & 7.6 Hz, 7-H), 6.87-7.43 (16H, m, PhO & $CHPh_2$) and 7.71 (1H, d, J=7.6 Hz, N-H); (Found; C,65.99; H,4.82; N,7.13; S,5.42. $C_{32}H_{27}N_3O_6S$ requires C,66.08; H,4.68; N,7.23; S,5.51%); m/e 599 MNH_4^+.

Synthesis of Diphenylmethyl (6R,7R) 2α-4β-Di-(3'-oxobutyl)-3-methyl-7β-phenoxyacetamidoceph-2-em-4α-carboxylate 1β-Oxide (383).

A suspension of the sulphoxide **(202)** (5g, 10 mmol) in methyl vinyl ketone (20 ml) with triethylamine (3.03g, 30 mmol) was stirred at room temperature for 19 hr. According to tlc starting material remained unreacted hence triethylamine (5 ml) was added and after 10 hr the Michael acceptor was evaporated *in vacuo*. The oily residue was dissolved in ethyl acetate and washed with dil HCl, brine, saturated aqueous $NaHCO_3$, brine; dried ($MgSO_4$) and concentrated *in vacuo*. Chromatography afforded ***diphenylmethyl (6R,7R) 2α,4β-di-(3'-oxobutyl)-3-methyl-7β-phenoxyacetamidoceph-2-em-4α-carboxylate 1β-oxide (383)*** as white crystals (1.68g, 32%); mp 119-120 °C; υ_{max} 3360, 1778, 1755 and 1713 cm^{-1}; δ (250 MHz) 1.66 (3H, s, 3-CH_3), 2.12 (3H, s, $COCH_3$), 2.14 (3H, s, $COCH_3$), 2.5-3.1 (8H, m, CH_2CH_2CO), 4.55 (2H, s, CH_2CON), 4.62 (1H, d, J=4.7 Hz, 6-H), 5.81 (1H, dd, J=4.7 & 10.5 Hz, 7-H), 6.8-7.4 (16H, m, PhO & $CHPh_2$) and 8.12 (1H, d, J=10.5 Hz, N-H); (Found: C,66.14; H,5.67; N,4.06; S,4.68. $C_{37}H_{38}N_2O_8S$ requires C,66.25; H,5.71; N,4.17; S,4.77%); m/e 671 MH^+.

Also isolated was ***diphenylmethyl (6R,7R) 3-methyl-4β-(3'-oxobutyl)-7β-phenoxyacetamidoceph-2-em-4α-carboxylate 1β-oxide (385)*** as crystals (0.78g, 22%); mp 175-177°C; υ_{max} 3354, 1779, 1741 and 1695 cm^{-1}; δ (250 MHz) 1.81 (3H, d, J=0.9 Hz, 3-CH_3), 2.15 (3H, s, $COCH_3$), 2.5-3.1 (4H, m, CH_2CH_2CO), 4.54 (1H, d, J=5.0 Hz, 6-H), 4.55 (2H, s, CH_2CON), 5.85 (1H, dd, J=4.9 & 10.5 Hz, 7-H), 6.72 (1H, d, J=0.9 Hz, 2-H), 6.91-7.39 (16H, m, PhO & $CHPh_2$) and 8.20 (1H, d, J=10.5 Hz, N-H); (Found: C,66.05; H,5.32; N,4.64; S,5.28. $C_{33}H_{32}N_2O_7S$ requires C,65.98; H, 5.37; N,4.66; S,5.34%); m/e 624 MNa^+.

Attempted Reaction of Diphenylmethyl (6R,7R) 3-Methyl-7β-phenoxy-acetamidoceph-3-em-4-carboxylate 1β-Oxide (202) with Diethyl Ethoxymethylenemalonate.

Triethylamine (0.2, 2 mmol) and diethyl ethoxymethylenemalonate (0.43g, 2 mmol) were added to a stirred solution of the sulphoxide **(202)** (0.5g, 1 mmol) in dry THF (25 ml). After 1.5 hr tlc indicated that the reaction mixture contained only unreacted starting material. More triethylamine (1 ml) was added and stirring continued for 1.5 hr after which time tlc still showed no trace of products. The solution was heated at reflux for 2 hr and the starting material **(202)** was still present as indicated by tlc. Thus reaction was abandoned.

Attempted Reaction of Diphenylmethyl (6R,7R) 3-Methyl-7β-phenoxy-acetamidoceph-3-em-4-carboxylate 1β-Oxide (202) with Diethyl Ethoxy-methylenemalonate.

To the sulphoxide (0.5g, 1 mmol) in dry THF (25 ml) was added diethyl ethoxymethylenemalonate (0.43g, 2 mmol) and Triton B (0.2 ml) and after 10 minutes, ir indicated the loss of the β-lactam carbonyl peak (υ_{max} ~1790 cm^{-1}) and therefore the reaction mixture was discarded.

Synthesis of Diphenylmethyl (6R,7R) 2-diethylmethylene malonate 3-Methyl-7β-phenoxyacetamidoceph-3-em-4-carboxylate 1β-Oxide (390).

The sulphoxide (2g, 4 mmol) was stirred in diethyl ethoxy-methylenemalonate (10 ml) at room temperature with triethylamine (1.21g, 12 mmol) for 15 hr. Ethyl acetate was added and the reaction mixture was washed with dil HCl, brine, saturated aqueous $NaHCO_3$ solution, brine, dried ($MgSO_4$) and the solvent removed *in vacuo*. Column chromatography afforded ***diphenylmethyl (6R,7R) 2-diethyl methylenemalonate-3-methyl-7β-phenoxyacetamido-ceph-3-em-4-carboxylate 1β-oxide (390)*** as a bright yellow oil (1.0g, 38%); υ_{max}

3369, 1794 and 1734 cm^{-1}; δ (250 MHz) 1.25-1.36 (6H, m, $CH_2C\underline{H}_3$), 2.2 (3H, s, 3-CH_3), 4.19-4.33 (4H, m, $C\underline{H}_2CH_3$), 4.60 (2H, s, CH_2CON), 4.83 (1H, d, J=4.8 Hz, 6-H), 4.86 (1H, d, J=9.3 Hz, $C\underline{H}(CO_2Et)_2$), 6.11 (1H, dd, J=4.8 & 10.4 Hz, 7-H), 6.67 (1H, d, J=9.3 Hz, 2-CH), 6.92-7.44 (16H, m, PhO & $CHPh_2$) and 7.88 (1H, d, J=10.4 Hz, N-H); (Found: C,62.99; H,4.82; N,3.95; S,4.26. $C_{37}H_{36}N_2O_{10}S$ requires C,63.41; H,5.18; N,4.00; S,4.58%); m/e 725 MNa^+ and 703 MH^+.

The second component isolated was ***diphenylmethyl (6R,7R) 2α-diethyl ethoxymethylmalonate-3-methyl-7β-phenoxyacetamidoceph-3-em-4-carboxylate 1β-oxide (391)*** (0.18g, 6%); υ_{max} 3364, 1791, 1735, 1651 cm^{-1}; δ (250 MHz) 1.25-1.33 (9H, m, $CH_2C\underline{H}_3$), 2.29 (3H, s, 3-CH_3) 3.36-3.48 (2H, m, $OC\underline{H}_2CH_3$), 3.82-3.89 (2H, m, 2-H), 4.25 (4H, m, $C\underline{H}_2CH_3$), 4.58 (2H, s, CH_2CON), 4.9 (1H, d, J=4.8 Hz, 6-H), 6.18 (1H, dd, J=4.8 & 9.6 Hz, 7-H), 6.95-7.51 (16H, m, PhO & $CHPh_2$) and 7.93 (1H, d, J=9.6 Hz, N-H); (Found: C,62.96; H,6.01; N,3.48; S,3.6. $C_{39}H_{42}N_2O_{11}S$ requires C,62.72; H,5.67; N,3.75; S,4.29%); m/e 769 MNa^+.

The last compound isolated by chromatography was ***diphenylmethyl (6R,7R) 2-ethoxy-3-methyl-7β-phenoxyacetamidoceph-3-em-4-carboxylate 1β-oxide (392)*** as white crystals (0.52g, 24%); mp 203-205°C; υ_{max} 3375, 1781 and 1696 cm^{-1}; δ (250 MHz) 1.38 (3H, t, J=7.0 Hz, $OCH_2C\underline{H}_3$), 2.40 (3H, s, 3-CH_3), 4.18 (2H, q, J=7.0 Hz, $OC\underline{H}_2CH_3$), 4.46 (1H, d, J=4.8 Hz, 6-H), 4.57 (2H, s, CH_2CON), 6.12 (1H, dd, J=4.8 & 10.4 Hz, 7-H), 6.89-7.42 (16H, m, PhO & $CHPh_2$), 8.06 (1H, d, J=10.4 Hz, N-H); (Found: C,64.72; H,5.09; N,4.74. $C_{31}H_{30}N_2O_7S$ requires C,64.79; H,5.26; N,4.88%); m/e 597 MNa^+ and 408.9 MH^+-DPM ester.

Attempted Elimination of Ethoxy group from Sulphoxide (391).

Triethylamine (0.5 ml) was stirred with a solution of the sulphoxide **(391)** (0.05g, 0.07 mmol) in dry THF (5 ml) for 24 hr. Starting material remained

unreacted according to tlc and therefore more triethylamine (1 ml) was added. After 24 hr tlc indicated no products were present and therefore the reaction solution was heated at reflux for 1 hr which resulted in a complex mixture of products, inseparable by column chromatography.

Preparation of Diphenylmethyl (6R,7R) 2-Ethoxy-3-Methyl-7β-phenoxy-acetamidoceph-3-em-4-carboxylate 1β-Oxide (392).

Trethylamine (0.88g, 9 mmol) was added to a suspension of the sulphoxide (1.54g, 3 mmol) in diethyl ethoxymethylenemalonate (15 ml) at room temperature. After 20 hr ethyl acetate was added and the solution was washed with 1M HCl, brine, saturated aqueous $NaHCO_3$, brine, dried ($MgSO_4$) and ethyl acetate removed *in vacuo*. Diethyl ethoxymethylenemalonate was removed by bulb to bulb distillation. Column chromatography afforded ***diphenylmethyl (6R,7R) 2-ethoxy-3-methyl-7β-phenoxyacetamidoceph-3-em 1β-oxide (392)*** (0.37g, 22%), identical (ir, nmr) to that obtained previously.

Preparation of Diphenylmethyl (6R,7R) 2-Diethyl Methylenemalonate-3-methyl-7β-phenoxyacetamidoceph-3-em-4-carboxylate 1β-oxide (390).

The sulphoxide (2g, 4 mmol) was stirred in diethyl ethoxy-methylenemalonate (10 ml) at room temperature with triethylamine (1.21g, 12 mmol) for 15 hr. Ethyl acetate was added and the reaction mixture was washed with dil HCl, brine, saturated aqueous $NaHCO_3$ solution, brine, dried ($MgSO_4$) and the solvent removed *in vacuo*. Column chromatography over a period of 4 days afforded ***diphenylmethyl (6R,7R) 2-diethyl methylenemalonate-3-methyl-7β-phenoxyacetamidoceph-3-em-4-carboxylate 1β-oxide (390)*** as a bright yellow oil (1.3g, 46%), identical to (ir, nmr) to that obtained previously.

Attempted Epimerisation of (202) in Acetonitrile.

A solution of the ester **(202)** (0.5g, 1 mmol) in acetonitrile (20 ml) with triethylamine (1.5 ml) was stirred at room temperature. The reaction was monitored over a period of 5 days during which time the majority of ester **(202)** remained unreacted and an increase in baseline material was observed (tlc) and hence, reaction mixture was discarded.

Attempted Michael Addition of (202) with Methyl Propiolate in dry THF.

Triethylamine (0.3g, 3 mmol) was added to a solution of **(202)** (0.5g, 1 mmol) and methyl propiolate (0.25g, 3 mmol) in dry THF and stirring was continued for 5 hr at room temperature. The solvent was evaporated under reduced pressure and a black oily residue was obtained. The ir spectrum contained no evidence for the β-lactam carbonyl and the oil was discarded.

Attempted Michael Addition of (202) with Methyl Propiolate using Acetonitrile as Solvent.

To an ice temperature solution of the sulphoxide **(202)** (0.5g, 1 mmol) in acetonitrile, methyl propiolate (0.25g, 3 mmol) and triethylamine (0.3g, 3 mmol) were added. The solution was stirred for 2 hr and washed with dilute HCl, brine, saturated aqueous $NaHCO_3$, brine, dried ($MgSO_4$) and concentrated *in vacuo* to an oil which gave a weak β-lactam carbonyl peak (cm^{-1}) according to ir. Chromatography afforded only one β-lactam product in less than 30mg which due to the low yield was discarded.

Attempted Michael Addition of (202) with 3-Ethoxymethacrolein in dry THF.

A solution of the sulphoxide **(202)** (0.1g, 2 mmol), triethylamine (0.06g, 0.6 mmol) and 3-ethoxymethacrolein (0.06g, 0.5 mmol) in dry THF (10 ml) was stirred at room temperature for 24 hr. According to tlc no reaction had taken place

and the reaction was abandoned.

Attempted Michael Addition of Sulphoxide (202) with Neat 3-Ethoxy-methacrolein.

Triethylamine (0.3g, 3 mmol) was added to a suspension of the sulphoxide (0.5g, 1 mmol) in 3-ethoxymethacrolein (15 ml) at room temperature. After 24 hr, three products were observed on tlc. Ethyl acetate (30 ml) was added and the phase washed with dil HCl, water, saturated aqueous $NaHCO_3$, brine; dried ($MgSO_4$) and the solvent evaporated *in vacuo* to give a gel. Attempts at dissolving the gel with ethyl acetate, dichloromethane and acetone proved fruitless and the products remained trapped within the gel.

Synthesis of Diphenylmethyl (6R,7R) 3-Methyl-4β-(2'-cyanoethyl)-7β-phenoxyacetamidoceph-2-em-4-carboxylate (362).

A solution of the sulphide **(361)** (5.13g, 10mmol) in acrylonitrile (40 ml) with triethylamine (2 ml) was stirred at room temperature for 20 hr. The solvent was removed *in vacuo* and the residue chromatographed to give ***diphenylmethyl (6R,7R) 3-methyl-4β-(2'-cyanoethyl)-7β-phenoxyacetamidoceph-2-em-4α-carboxylate (362)*** as a colourless oil (2.8g, 49%); υ_{max} 3390, 2251, 1788, 1740 and 1700 cm^{-1}; δ (250 MHz) 1.55 (3H, d, J=1.2 Hz, 3-CH_3), 1.99-2.50 (3H, m, 4-$C\underline{H}_2C\underline{H}_2CN$), 2.97-3.18 (1H, m, 4-$CH_2CH_2CN$), 4.24 (2H, s, CH_2CON), 4.48 (1H, d, J=4.8 Hz, 6-H), 5.64 (1H, dd, J=4.8 & 9.6 Hz, 7-H), 6.23 (1H, d, J=1.2 Hz, 2-H), 6.49-7.25 (16H, m, PhO & $CHPh_2$) and 7.80 (1H, d, J=9.6 Hz, N-H); (Found: C,67.58; H,4.95; N,7.32; S,5.47. $C_{32}H_{29}N_3O_5S$ requires C,67.72; H,5.11; N,7.41, S,5.64%); m/e 590 MNa^+.

Oxidation of 4β-(2'-cyanoethyl) Adduct (362).

A solution of the sulphide **(362)** (2.8g, 5 mmol) in dichloromethane (20 ml) was stirred with *m*-CPBA (1.92g, 5.5 mmol of 50%) for 1 hr. The reaction mixture was washed with 10% $Na_2S_2O_5$, brine, saturated aqueous $NaHCO_3$, brine, dried ($MgSO_4$), evaporated *in vacuo* and chromatographed on silica gel to give ***diphenylmethyl (6R,7R) 3-methyl-4β-(2'-cyanoethyl)-7β-phenoxyacetamido ceph-2-em-4α-carboxylate 1β-oxide (402)*** as white crystals (2.53g, 87%); mp 159-160^{0}C; υ_{max} 3372, 2250, 1783, 1743 and 1687 cm^{-1}; δ (250 MHz) 1.67 (3H, d, J=1.2 Hz, 3-CH_3), 2.00-2.80 (3H, m, 4-$C\underline{H}_2C\underline{H}_2CN$), 3.14-3.29 (1H, m, 4-$CH_2CH_2CN$), 4.24 (2H, s, CH_2CON), 4.28 (1H, d, J=4.8 Hz, 6-H), 5.87 (1H, dd, J=4.8 & 9.6 Hz, 7-H), 6.42 (1H, d, J=1.2 Hz, 2-H), 6.65-7.43 (16H, m, PhO & $CHPh_2$) and 8.01 (1H, d, J=9.6 Hz, N-H); (Found: C,66.17; H,4.72; N,4.89; S,5.63. $C_{32}H_{29}N_3O_6S$ requires C,65.87; H,4.97; N,7.20; S,5.49); m/e 607 MNa^+.

Attempted Retro-Michael Reaction of Diphenylmethyl (6R,7R) 3-Methyl-4β-(2'-cyanoethyl)-7β-phenoxyacetamidoceph-2-em-4α-carboxylate 1β-Oxide (402) using Triethylamine.

Triethylamine (0.053g, 0.51 mmol) was added to a stirred solution of the sulphoxide **(402)** (0.1g, 0.17 mmol) in dry THF. After 24 hr tlc analysis indicated starting material remained unreacted. The reaction solution was refluxed for 3 hr, however, according to tlc the starting material **(402)** was still present and therefore the reaction was abandoned.

Attempted Retro-Michael Reaction of Diphenylmethyl (6R,7R) 3-Methyl-4β-(2'-cyanoethyl)-7β-phenoxyacetamidoceph-2-em-4α-carboxylate 1β-Oxide (402) using Sodium Hydride.

Sodium hydride (0.01g, 0.34 mmol) was stirred with the sulphoxide **(402)** (0.1g, 0.17 mmol) in dry THF at 0°C. After 24 hr, products were observed

on tlc as well as some unreacted starting material therefore the THF solvent was evaporated *in vacuo* and the residue was dissolved in ethyl acetate. The reaction solution was poured onto a mixture of ice/water and the organic layer separated, dried ($MgSO_4$) and concentrated under reduced pressure. The ir spectrum indicated the presence of a β-lactam ring, however pressurised column chromatography failed as the products were too numerous to isolate and the reaction was abandoned.

Attempted Retro-Michael Reaction of Diphenylmethyl (6R,7R) 3-Methyl-2,2-di-(2'-cyanoethyl)-7β-phenoxyacetamidoceph-3-em-4-carboxylate 1β-Oxide (373) using Triethylamine.

The ester **(373)** (0.4g, 0.63mmol) and triethylamine (0.2g, 1.9 mmol) were stirred in dry THF for 24 hr, after which time tlc indicated the presence of unreacted starting material. An excess of triethylamine (5 ml) was added and again after 24 hr the ester **(373)** remained unreacted according to tlc. The solution was then refluxed for 2 hr and evaporated *in vacuo* after tlc revealed the presence of numerous products. A light brown oily residue was obtained which according to ir did not display a β-lactam carbonyl stretching frequency and therefore only contained degradation products. Thus, reaction mixture was discarded.

Attempted Retro-Michael Reaction of Diphenylmethyl (6R,7R) 3-Methyl-2,2-di-(2'-cyanoethyl)-7β-phenoxyacetamidoceph-3-em-4-carboxylate 1β-Oxide (373) using Sodium Hydride.

The sulphoxide **(373)** (0.5g, 0.8 mmol) and sodium hydride (0.05, 2.1 mmol) in dry THF (30 ml) were refluxed for 4 hr after which time products were observed according to tlc. The reaction mixture was poured onto a mixture of ice and water; extracted with ethyl acetate; dried ($MgSO_4$) and evaporated *in vacuo* to give an oil. Column chromatography was attempted but failed as a result of the

numerous products present in the reaction mixture and so reaction was abandoned.

Attempted Retro-Michael Reaction of Diphenylmethyl (6R,7R) 3-Methyl-2,2-di-(2'-cyanoethyl)-7β-phenoxyacetamidoceph-3-em-4-carboxylate 1β-Oxide (373) using Sodium Hydride.

Sodium hydride (0.03g, 1.3 mmol) was added to a solution of **(373)** (0.4g, 0.63 mmol) in DMF under a blanket of nitrogen at 0°C. After 24 hr, the reaction mixture was poured onto water containing ice; extracted with ethyl acetate; dried ($MgSO_4$) and concentrated under reduced pressure to afford a brown oil that contained no β-lactam ring carbonyl according to ir and so reaction mixture was discarded.

Attempted Retro-Michael Reaction of Diphenylmethyl (6R,7R) 3-Methyl-2,2-di-(2'-cyanoethyl)-7β-phenoxyacetamidoceph-3-em-4-carboxylate 1β-Oxide (373) using Triethylamine.

Triethylamine (1.25g, 12 mmol) was added to a stirred solution of the di-adduct **(373)** (2.62g, 4 mmol) in acrylonitrile (30 ml) for 48 hr. Acrylonitrile was evaporated *in vacuo* and the oily residue dissolved in ethyl acetate; washed with dil HCl, water, saturated aqueous $NaHCO_3$, brine; dried ($MgSO_4$) and concentrated *in vacuo*. After chromatography three products were obtained and starting material (0.97g) was recovered. According to ir no peak for the β-lactam carbonyl group was observed in any of the three minor components so the reaction was abandoned.

Attempted Retro-Michael Reaction of Diphenylmethyl (6R,7R) 3-Methyl-4β-(3'-oxobutyl)-7β-phenoxyacetamidoceph-2-em-4α-carboxylate 1β-Oxide (385) using Triethylamine.

The sulphoxide **(385)** (1g, 1.67 mmol) was dissolved in dry THF (35 ml) at room temperature. Triethylamine (0.51g, 5 mmol) was added to this solution and stirring was continued for 20 hr. According to tlc the starting material remained unreacted therefore more triethylamine (2 ml) was added and the mixture was refluxed for 3 hr. Tlc indicated no reaction had taken place and the sulphoxide **(385)** was still present therefore reaction was not investigated further.

Attempted Retro-Michael Reaction of Diphenylmethyl (6R,7R) 3-Methyl-2α,4β-di-(3'-oxobutyl)-7β-phenoxyacetamidoceph-2-em-4α-carboxylate 1β-Oxide (383) using Triethylamine.

The di-adduct **(383)** (0.18g, 0.27 mmol) was dissolved in toluene (10 ml) and heated at reflux with triethylamine (1 ml) for 4 hr after which time tlc indicated no elimination had taken place and starting material was recovered unreacted.

Attempted Retro-Michael Reaction of Diphenylmethyl (6R,7R) 3-Methyl-2α,4β-di-(3'-oxobutyl)-7β-phenoxyacetamidoceph-2-em-4α-carboxylate 1β-Oxide (383) using Sodium Hydride.

Sodium hydride (0.01g, 0.4 mmol) was added to a solution of the sulphoxide **(383)** (0.2g, 0.3 mmol) in toluene and the reaction mixture was heated at reflux for 1 hr. Tlc indicated the majority of starting material had been converted to baseline material as a result of degradation according to ir which did not display the β-lactam carbonyl stretching frequency. Hence, reaction was abandoned.

Attempted Retro-Michael Reaction of Diphenylmethyl (6R,7R) 3-Methyl-2α,4β-di-(3'-oxobutyl)-7β-phenoxyacetamidoceph-2-em-4α-carboxylate 1β-Oxide (383) using p-Toluene Sulphonic Acid.

The di-adduct **(383)** (0.2g, 0.3 mmol) was dissolved in toluene (10 ml) and heated at reflux with *p*-toluene sulphonic acid. Reaction mixture appeared as a streak with a lot of baseline material according to tlc therefore it was discarded.

Attempted Reaction of Diphenylmethyl (6R,7R) 3-Methyl-7β-phenoxy-acetamidoceph-3-em-4-carboxylate 1,1-dioxide (417) with diethyl ethoxy-methylenemalonate.

The sulphone **(417)** (0.52g, 1 mmol) and triethylamine (0.3g, 3mmol) were stirred with diethyl ethoxymethylenemalonate at room temperature overnight. Triethylamine was removed under vacuum and ir indicated loss of the β-lactam carbonyl peak (~1785 cm^{-1}) and reaction was abandoned.

Attempted Reaction of Diphenylmethyl (6R,7R) 3-Methyl-7β-phenoxy-acetamidoceph-3-em-4-carboxylate 1β-Oxide (202) with Benzyl Bromide.

The sulphoxide **(202)** (0.56g, 1.1mmol) was dissolved in DMF (35 ml) under a nitrogen blanket. The solution was cooled to 0°C and sodium hydride (0.03g, 1.3 mmol) was added followed by benzyl bromide (0.23g, 1.3 mmol). After 3 hr, tlc indicated the majority of starting material had reacted. The reaction mixture poured onto ice/water; extracted with ethyl acetate; washed with dil HCl, brine, saturated aqueous $NaHCO_3$, brine; dried ($MgSO_4$) and concentrated *in vacuo* to give a red oil (1.0g). Chromatography afforded an inseparable mixture of two components and sulphoxide **(202)** (0.18g).

Attempted Reaction of Diphenylmethyl (6R,7R) 3-Methyl-7β-phenoxy-acetamidoceph-3-em-4-carboxylate 1β-Oxide (202) with Benzyl Bromide.

A suspension of sodium hydride (0.06g, 2.6 mmol) in DMSO (40 ml) was stirred at room temperature. The sulphoxide **(202)** (1.0g, 2.2 mmol) dissolved in DMSO (30 ml) with benzyl bromide (0.44g, 2.6 mmol) was added to this solution dropwise and stirring continued for 3 hr. The reaction mixture was poured onto ice/water; extracted with ethyl acetate; washed with dil HCl, brine, saturated aqueous $NaHCO_3$, brine; dried ($MgSO_4$) and evaporated *in vacuo*. A red-brown oil (1.05g) was obtained which contained a complex mixture of products inseparable by column chromatography and was therefore discarded.

Synthesis of Diphenylmethyl (6R,7R) 2α,4β-Dibenzyl-3-Methyl-7β-phenoxy-acetamidoceph-3-em-4-carboxylate 1β-Oxide (431).

The sulphoxide **(202)** (5.0g, 10 mmol) was dissolved in DMF (50 ml) under a blanket of nitrogen and cooled to 0°C. Benzyl bromide (2.1g, 12 mmol) was added followed by sodium hydride (0.16g, 6.6 mmol). A second portion of sodium hydride (0.14g, 5.8 mmol) was added after 30 minutes. Stirring was continued for 15 hr and the solution was poured onto ice/water; extracted with ethyl acetate; washed with dil HCl, saturated aqueous $NaHCO_3$, brine; dried ($MgSO_4$) and concentrated under vacuum. Column chromatography afforded ***diphenylmethyl (6R,7R) 2α,4β-dibenzyl-3-methyl-7β-phenoxyacetamido-ceph-2-em-4-carboxylate 1β-oxide (431)*** as a white foam (0.62g, 10%); υ_{max} 3648, 1780, 1737 and 1693 cm^{-1}; δ (250 MHz) 1.38 (3H, s, 3-CH_3), 3.95-4.2 (4H, m, 2-CH_2Ph & 4-CH_2Ph), 4.41 (1H, d, J=4.6 Hz, 6-H), 4.44 & 4.51 (2H, ABq, J=15.4 Hz, CH_2CON), 5.73 (1H, dd, J=4.6 & 10.5 Hz, 7-H), 6.91-7.44 (26H, m, PhO, $CHPh_2$, 2-CH_2Ph & 4-$CHPh_2$) and 8.03 (1H, d, J=10.5 Hz, N-H); (Found: C,72.48; H,5.49; N,3.66; S,4.35. $C_{43}H_{38}N_2O_6S$ requires C,72.56; H,5.39; N,3.94; S,4.51%); m/e 733 MNa^+.

A mixture of di-adduct **(431)** (2.49g) was also contaminated with another component according to tlc.

Preparation of Diphenylmethyl (6R,7R) 2α-Diphenylmethyl-3-methyl-7β-phenoxyacetamidoceph-3-em-4-carboxylate 1β-Oxide (435).

The sulphoxide **(202)** (5.3g, 10 mmol) was dissolved in DMF (35 ml) and cooled to 0°C under a nitrogen blanket. Bromodiphenylmethane (4.97g, 20 mmol) was added, followed by sodium hydride (0.3g, 12 mmol). After 15 hr, the reaction mixture was poured onto ice/water and extracted with ethyl acetate. The organic phase was washed with dil HCl, brine, saturated aqueous $NaHCO_3$, brine; dried ($MgSO_4$); evaporated *in vacuo* and chromatographed to afford ***diphenylmethyl (6R,7R) 2α,2β-di(diphenylmethyl)-3-methyl-7β-phenoxy-acetamidoceph-3-em-4α-carboxylate 1β-oxide (434)*** as an oil (1.81g, 21%); υ_{max} 3370, 1799 and 1730 cm^{-1}; δ (250 MHz) 1.80 (3H, s, 3-CH_3), 3.4 (1H, d, J=4.5 Hz, 6-H), 4.44 & 4.50 (2H, ABq, J=15 Hz*, CH_2CON), 5.15 (1H, s, 2-C<u>H</u>Ph_2), 5.38 (1H, s, 2-C<u>H</u>Ph_2), 5.74 (1H, dd, J=4.6 & 10.5 Hz, 7-H), 6.73-7.5 (36H, m, PhO, $CHPh_2$, 2-$CHPh_2$ & 4-$CHPh_2$) and 8.14 (1H, d, J=10.5 Hz, N-H); (Found: C,76.29; H,5.42; N,3.11; S,3.7. $C_{55}H_{46}N_2O_6S$ requires C,76.54; H,5.37; N,3.25; S,3.72%); m/e 863 MH^+ and ***diphenylmethyl (6R,7R) 2α-diphenylmethyl-3-methyl- 7β-phenoxyacetamidoceph-3-em-4-carboxylate 1β-oxide (435)*** as an oil (1.79g, 26%); υ_{max} 3382, 1801 and 1730 cm^{-1}; δ (250 MHz) 1.65 (3H, s, 3-CH_3), 3.93 (1H, d, J=8.5 Hz, 2-H), 3.95 (1H, d, J=4.8 Hz, 6-H), 4.39 (1H, d, J=8.5 Hz, 2-C<u>H</u>Ph_2), 4.49 (2H, s, CH_2CON), 6.03 (1H, dd, J=4.8 & 10.6 Hz, 7-H), 6.9-7.4 (26H, m, PhO, $CHPh_2$ & 2-$CHPh_2$) and 7.87 (1H, d, J=10.6 Hz, N-H); (Found: C, 72.40; H,5.2; N,3.8; S,4.45. $C_{42}H_{36}N_2O_6S$ requires C,72.39; H,5.21; N,4.02; S,4.6%); m/e 697 MH^+ and 7.14 MNH_4^+.

Synthesis of Diphenylmethyl (6R,7R) 2α-t-Butylacetoxy-3-methyl-7β-phenoxyacetamidoceph-3-em-4-carboxylate 1β-Oxide (436).

A solution of the sulphoxide **(202)** (2.02g, 4 mmol) in DMF (30 ml) with *t*-butylbromoacetate (0.89g, 4.8 mmol) and sodium hydride (0.11g, 4.8 mmol) was stirred for 1 hr. The solution was poured onto ice/water and extracted with ethyl acetate. The organic portion was washed with dil HCl, brine, saturated aqueous $NaHCO_3$, brine; dried ($MgSO_4$) and evaporated *in vacuo* to give an oil which on recrystallisation from ethyl acetate/petrol afforded ***diphenylmethyl (6R,7R) 2α-t-butylacetoxy-3-methyl-7β-phenoxyacetamidoceph-3-em-4-carboxylate 1β-oxide (436)*** as light brown crystals (1.18, 48%); mp 165-167°C; υ_{max} 3334, 1801, 1727 and 1695 cm^{-1}; δ (60 MHz) 1.27 (9H, s, CMe_3), 1.88 (3H, s, 3-CH_3), 2.01 (1H, dd, J=7.2 & 15.6 Hz, 2-CH_2CO_2), 2.42 (1H, dd, J=3.8 & 15.6 Hz, 2-CH_2CO_2), 3.8 (1H, dd, J=3.8 & 7.2, 2-H), 4.25 (1H, d, J=4.8 Hz, 6-H), 4.28 (2H, s, CH_2CON), 5.88 (1H, dd, J=4.8 & 9.6 Hz, 7-H), 6.48-7.29 (16H, m, PhO & $CHPh_2$) and 7.65 (1H, d, J=9.6 Hz, N-H).

Synthesis of Diphenylmethyl (6R,7R) 4α-Allyl-3-methyl-7β-phenoxy-acetamidoceph-2-em-4β-carboxylate 1β-Oxide (441).

The sulphoxide **(202)** (1.14g, 2 mmol) dissolved in DMF (20 ml) at 0°C was stirred with sodium hydride (0.06g, 2.49 mmol) and allyl bromide (0.3g, 2.49 mmol) overnight. According to tlc some of the sulphoxide remained unreacted, hence more sodium hydride (0.06g, 2.49 mmol) and allyl bromide (0.3g, 2.49 mmol) were added and stirring continued for another 1.5 hr. The solution was poured onto ice/water and extracted with ethyl acetate. The organic portion was washed with dil HCl, brine, saturated aqueous $NaHCO_3$, brine; dried ($MgSO_4$) and evaporated *in vacuo* to give an oil. Chromatography on silica gel gave ***diphenylmethyl (6R,7R) 3-methyl-4α-allyl-7β-phenoxyacetamidoceph-2-em-4β-carboxylate 1β-oxide (441)*** as light yellow crystals (0.28g, 24%); mp

143-144°C; υ_{max} 3362, 1778, 1742 and 1690 cm^{-1}; δ (250 MHz) 1.78 (3H, s, 3-CH_3), 2.85-2.95 (1H, m, 4-C$\underline{H}_2$CHCH_2), 3.82-3.88 (1H, m, 4-C$\underline{H}_2$CHCH_2), 4.53 & 4.59 (2H, ABq, J=15.2 Hz*, CH_2CON), 4.59 (1H, d, J=4.8 Hz, 6-H), 5.14-5.20 (2H, m, 4-CH_2CHC$\underline{H}_2$), 5.86 (1H, dd, J=4.8 & 10.4 Hz, 7-H), 5.93-6.01 (1H, m, 4-CH_2C$\underline{H}$CH_2), 6.70 (1H, s, 2-H), 6.94-7.40 (16H, m, PhO & CHPh_2) and 8.27 (1H, d, J=10.4 Hz, N-H); (Found: C,67.15; H,5.49; N,4.86; S,5.54. $C_{32}H_{30}N_2O_6S$ requires C,67.35; H,5.30; N,4.91; S,5.62%); m/e 571 MH^+.

Also isolated was ***diphenylmethyl (6R,7R) 2,4β-diallyl-3-methyl-7β-phenoxyacetamidoceph-2-em-4α-carboxylate 1β-oxide (442)*** as a crystalline solid (0.16g, 13%); mp 126-128°C; υ_{max} 3375, 1781, 1739 and 1694 cm^{-1}; δ (250 MHz) 1.64 (3H, s, 3-CH_3), 2.83-2.93 (1H, m, 4-C$\underline{H}_2$CHCH_2) 3.24-3.27 (2H, m, 2-C$\underline{H}_2$CHCH_2), 3.81-3.89 (1H, m, 4-C$\underline{H}_2$CHCH_2), 4.53 (2H, s, CH_2CON), 4.56 (1H, d, J=4.7 Hz, 6-H), 4.98-5.16 (4H, m, 2-CH_2CHC$\underline{H}_2$ & 4-CH_2CHC$\underline{H}_2$), 5.68-5.87 (3H, m, 7-H, 2-CH_2C$\underline{H}$CH_2 & 4-CH_2C$\underline{H}$CH_2), 6.92-7.41 (16H, m, PhO & CHPh_2) and 8.18 (1H, d, J=10.5 Hz, N-H); (Found: C,69.02; H,5.33; N,4.87; S,4.98. $C_{35}H_{34}N_2O_6S$ requires C,68.85; H,5.57; N,4.59; S,5.25); m/e 611 MH^+.

Preparation of Diphenylmethyl (6R,7R) 2α,2β-(3',3'- Dimethylallyl 3-methyl-7β-phenoxyacetamidoceph-3-em-4-carboxylate 1β-Oxide (448).

Sodium hydride (0.3g, 12 mmol) and 1-bromo-3-methylbutene (1.8g, 12 mmol) were added to a solution of the sulphoxide **(202)** (5g, 10 mmol) in DMF (50 ml) at 0°C in a nitrogen atmosphere. Stirring was continued for 15 hr after which time tlc indicated starting material remained unreacted, hence sodium hydride (0.17g, 7 mmol) and 1-bromo-3-methylbutene (1.8g, 12mmol) were added. After 3 hr stirring at room temperature, reaction mixture was poured onto ice/water; extracted with ethyl acetate; washed with dil HCl, brine, saturated aqueous $NaHCO_3$, brine; dried ($MgSO_4$) and concentrated under vacuum to give a brown oil. Chromatography isolated one product which was identified as ***diphenylmethyl***

(6R,7R) 2α,2β-di(3',3'-dimethylallyl)-3-methyl-7β-phenoxyacetamido-ceph-3-em-4-carboxylate 1β-oxide (448) as an oil (0.08g, 1.2%); υ_{max} 3350, 1782, 1738 and 1693 cm^{-1}; δ (250 MHz) 1.60 (6H, s, Me_2C=C), 1.71 (6H, s, Me_2C=C), 1.75 (3H, s, 3-CH_3), 2.95-3.62 (4H, m, $C\underline{H}_2CH$=CMe_2), 4.50 (1H, d, J=4.9 Hz, 6-H), 4.53 (2H, s, CH_2CON), 4.92-5.02 (1H, m, $CH_2C\underline{H}$=CMe_2), 5.29-5.31 (1H, m, $CH_2C\underline{H}$=CMe_2), 5.79 (1H, dd, J=4.8 & 10.5 Hz, 7-H), 6.90-7.03 (16H, m, PhO & $CHPh_2$), 8.23 (1H, d, J=10.5 Hz, N-H); (Found: C,70.19; H,6.26; N,4.09; S,4.84. $C_{39}H_{42}N_2O_6S$ requires C,70.24; H,6.35; N,4.20; S,4.81%); m/e 668 MH^+.

Preparation of Diphenylmethyl (6R,7R) 2α-Bromo-3-methyl-7β-phenoxy-acetamidoceph-3-em-4-carboxylate 1β-Oxide (450).

The sulphoxide **(202)** (1.0g, 1.9 mmol) was dissolved in DMF and cooled to 0°C under a nitrogen blanket. Sodium hydride (0.06g, 2.5 mmol) was added followed by bromodiethyl malonate (0.5g, 2.1 mmol) and stirring continued for 45 minutes. The reaction solution was poured onto ice/water and extracted with ethyl acetate. The organic layer was washed with dil HCl, brine, saturated aqueous $NaHCO_3$, brine; dried ($MgSO_4$) and evaporated *in vacuo*. Column chromatography isolated one product from the complex reaction mixture which was identified as ***diphenylmethyl (6R,7R) 2-bromo-3-methyl-7β-phenoxy-acetamidoceph-3-em-4-carboxylate 1β-oxide (450)*** as an oil (0.36g, 31%); υ_{max} 3383, 1801, 1731 and 1699 cm^{-1}; δ (60 MHz) 2.19 (3H, s, 3-CH_3), 4.75 (1H, s, 2-H), 5.09 (2H, s, CH_2CON), 5.18 (1H, d, J=4.8 Hz, 6-H), 6.20 (1H, dd, J=4.8 & 9.6 Hz, 7-H), 6.8-7.4 (16H, m, PhO & $CHPh_2$) and 7.55 (1H, d, J= 9.6 Hz, N-H).

Preparation of Diphenylmethyl (6R,7R) 3-Methyl-4β-allyl-7β-phenoxy-acetamidoceph-3-em-4α-carboxylate (452).

A solution of the sulphide **(361)** (1.0g, 2 mmol) in DMF (20 ml) was stirred overnight at 0°C under a nitrogen atmosphere with sodium hydride (0.06g, 2.53 mmol) and allyl bromide (0.31g, 2.53 mmol). The reaction mixture was poured onto ice/water; extracted with ethyl acetate; washed with dil HCl, brine, saturated aqueous $NaHCO_3$, brine; dried ($MgSO_4$) and evaporated under reduced pressure. Chromatography on silica gel afforded ***diphenylmethyl (6R,7R) 3-methyl-4β-allyl-7β-phenoxyacetamidoceph-2-em-4α-carboxylate (452)*** as a yellow oil (0.84g, 78%); υ_{max} 3305, 1778, 1745 and 1693 cm^{-1}; δ (250 MHz) 1.74 (3H, d, J=0.78 Hz, 3-CH_3), 2.89-3.02 (1H, m, 4-$C\underline{H}_2CHCH_2$), 3.41-3.55 (1H, m, 4-$C\underline{H}_2CHCH_2$), 4.53 (1H, s, CH_2CON), 5.04-5.27 (2H, m, 4-$CH_2CHC\underline{H}_2$), 5.18 (1H, d, J=4.6 Hz, 6-H), 5.49 (1H, dd, J=4.6 & 8.9 Hz, 7-H), 5.54-5.78 (1H, m, 4-$CH_2C\underline{H}CH_2$), 6.10 (1H, d, J=0.78 Hz, 2-H) and 6.85-7.41 (17H, m, PhO, $CHPh_2$ & N-H); (Found: C,69.81; H,5.53; N,4.70; S,5.48. $C_{32}H_{30}N_2O_5S$ requires C,69.29; H,5.45; N,5.05; S,5.78%); m/e 555 MH^+, 577.1 MNa^+ and 663 $[M+thioglycerol]^+$.

Oxidation of Diphenylmethyl (6R,7R) 3-Methyl-4β-allyl-7β-phenoxy-acetamidoceph-2-em-4α-carboxylate (452) with* m-*CPBA.

m-CPBA (0.28g, 1.4 mmol of 85%) dissolved in dichloromethane (10 ml) was added to a solution of the sulphide **(452)** (0.76g, 1.3 mmol) in dichloromethane (50 ml) at 0°C. Stirring was continued for 1.5 hr and dichloromethane (150 ml) added. The solution was washed with 10% $Na_2S_2O_5$, brine, saturated aqueous $NaHCO_3$, brine, dried ($MgSO_4$) and evaporated *in vacuo*. Chromatography isolated ***diphenylmethyl (6R,7R) 3-methyl-4β-allyl-7β-phenoxyacetamidoceph-2-em-4α-carboxylate 1β-oxide (453)*** as white crystals (0.4g, 51%); mp 141-142°C; υ_{max} 3351, 1780, 1739 and 1692 cm^{-1}; δ (250 MHz)

1.73 (3H, d, J=0.95 Hz, 3-CH_3), 2.82-2.92 (1H, m, 4-C$\underline{H}_2$CH=CH_2), 3.71-3.80 (1H, m, 4-C$\underline{H}_2$CH=CH_2), 4.50 & 4.57 (2H, ABq, J=15.2 Hz*, CH_2CON), 4.74 (1H, d, J=4.8 Hz, 6-H), 5.19-5.31 (2H, m, CH_2CH=C$\underline{H}_2$), 5.67-5.74 (1H, m, CH_2C$\underline{H}$=CH_2), 5.89 (1H, dd, J=4.8 & 10.7 Hz, 7-H), 6.33 (1H, d, J=0.96 Hz, 2-H), 6.91-7.35 (16H, m, PhO & $CHPh_2$) and 8.16 (1H, d, J=10.7 Hz, N-H); (Found: C,67.30; H,5.36; N,5.00; S,5.57. $C_{32}H_{30}N_2O_6S$ requires C,67.37; H,5.26; N,4.91; S,5.61%); m/e 571 MH^+ and 588 MNH_4^+.

Preparation of Diphenylmethyl (6R,7R) 3-Methyl-4β-allyl-7β-phenoxy-acetamidoceph-3-em-4α-carboxylate (452) Followed by Direct Oxidation with* m-*CPBA.

The sulphide **(361)** (1g, 2 mmol), sodium hydride (0.06g, 2.53 mmol) and allyl bromide (0.31g, 2.53 mmol) were stirred together overnight in DMF (20 ml) at 0°C under a blanket of nitrogen. The reaction mixture was partitioned between ethyl acetate and ice/water and the organic phase washed with dil HCl, brine, saturated aqueous $NaHCO_3$, brine, dried ($MgSO_4$) and evaporated under reduced pressure to give an oil (1.32g, 2.4 mmol) which was oxidised with *m*-CPBA (0.61g, 2.9 mmol of 80%) in dichloromethane. The resultant oil was chromatographed to give ***diphenylmethyl (6R,7R) 3-methyl-4β-allyl-7β-phenoxyacetamidoceph-2-em 4α-carboxylate 1β-oxide (453)*** as white crystals (0.44g, 40%). Spectroscopic and microanalysis data as before.

Attempted Cope Rearrangement of Diphenylmethyl (6R,7R) 3-Methyl-4β-allyl-7β-phenoxyacetamidoceph-2-em-4α-carboxylate (452).

The sulphide **(452)** (0.23g, 0.42 mmol) dissolved in toluene (10 ml) was heated at reflux for 6 hr. Tlc indicated starting material remained unreacted.

Attempted Cope Rearrangement of Diphenylmethyl (6R,7R) 3-Methyl-4β-allyl-7β-phenoxyacetamidoceph-2-em-4α-carboxylate (452).

The sulphide **(452)** (0.28g, 0.5 mmol) dissolved in toluene (20 ml) was oxidised with *m*-CPBA (0.12g, 0.54 mmol of 85%). After 1 hr the solution was heated at reflux for 3 hr after which time tlc indicated reaction mixture was too complex for separation by chromatography and was therefore abandoned.

Attempted Cope Rearrangement of Diphenylmethyl (6R,7R) 3-Methyl-4β-allyl-7β-phenoxyacetamidoceph-2-em-4α-carboxylate 1β-Oxide (441).

The sulphoxide **(441)** (0.1g, 0.2 mmol) was dissolved in toluene (10 ml) and refluxed for 5 hr. According to tlc, the sulphoxide **(441)** remained unreacted.

Attempted Cope Rearrangement of Diphenylmethyl (6R,7R) 3-Methyl-4β-allyl-7β-phenoxyacetamidoceph-2-em-4α-carboxylate 1β-Oxide (441).

The sulphoxide **(441)** (0.5g, 0.9 mmol) dissolved in xylene (20 ml) was heated 150-160°C using an oil bath for 1 hr and according to the ir the β-lactam ring had degraded so the reaction was abandoned.

Attempted Cope Rearrangement of Diphenylmethyl (6R,7R) 3-Methyl-4β-allyl-7β-phenoxyacetamidoceph-2-em-4α-carboxylate 1β-Oxide (441).

The sulphoxide **(441)** (0.5g, 1 mmol) was dissolved in xylene (10 ml) and heated at reflux for 6 hr. As indicated by tlc no reaction had occurred and starting material was recovered unreacted.

Preparation of Diphenylmethyl (6R,7R) 3-Methyl-4β-cyanomethyl-7β-phenoxy-acetamidoceph-3-em-4α-carboxylate 1β-Oxide (459) using Chloroacetonitrile.

The sulphoxide **(202)** (1.59g, 3 mmol) was dissolved in DMF (30 ml) and cooled to 0°C under a nitrogen atmosphere. Sodium hydride (0.11g, 4.5 mmol) and chloroacetonitrile (0.34g, 4.5 mmol) were added together. After 1.5 hr tlc indicated starting material remained unreacted therefore more sodium hydride (0.1g) was added followed by chloroacetonitrile (2 ml). After 1.5 hr reaction mixture was poured onto ice/water and extracted with ethyl acetate. The organic phase was washed with dil HCl, saturated aqueous $NaHCO_3$, brine; dried ($MgSO_4$) and concentrated *in vacuo*. Column chromatography afforded ***diphenylmethyl (6R,7R) 3-methyl-4β-cyanomethyl-7β-phenoxyacetamidoceph-2-em-4α-carboxylate 1β-oxide (459)*** as a white solid (0.36g, 21%); mp 146-147°C; υ_{max} 3352, 2218, 1786, 1741 and 1700 cm^{-1}. δ (250 MHz) 1.92 (3H, d, J=0.96 Hz, 3-CH_3), 3.72 & 3.88 (2H, ABq, J=32 Hz*, 4-CH_2CN), 4.55 (2H, s, CH_2CON), 4.58 (1H, d, J=4.85 Hz, 6-H), 5.92 (1H, dd, J=4.8 & 9.6 Hz, 7-H), 6.89-7.41 (17H, m, 2-H, PhO & $CHPh_2$) and 8.10 (1H, d, J=9.6 Hz, N-H); (Found: C,65.22; H,4.72; N,7.39; S,5.53. $C_{31}H_{27}N_3O_6S$ requires C,65.37; H,4.74; N,7.38; S,5.62%); m/e 569 M^+ and 593 $MNa^{+\cdot}$

Starting material **(202)** (0.4g) and an inseparable mixture of **(202)** and **(459)** were also recovered

Attempted Reaction of Diphenylmethyl (6R,7R) 3-Methyl-7β-phenoxy-acetamidoceph-3-em-4-carboxylate 1β-Oxide (202) with Bromoacetonitrile.

A solution of the sulphoxide (1g, 2 mmol) in acetonitrile (30 ml) at ice temperature was stirred with triethylamine (0.57g, 5.7 mmol) and bromoacetonitrile (0.68g, 5.7 mmol) for 5 hr after which time tlc indicated no reaction had taken place. Triethylamine (0.57g, 5.7 mmol) and bromoacetonitrile (0.68g, 5.7 mmol) were stirred into reaction mixture at room temperature. After 15

hr the build up of baseline material was observed on tlc but ir contained no β-lactam carbonyl stretching frequency and the reaction mixture was discarded.

Attempted Reaction of Diphenylmethyl (6R,7R) 3-Methyl-7β-phenoxy-acetamidoceph-3-em-4-carboxylate 1β-Oxide (202) with Bromoacetonitrile.

Sodium hydride (0.09g, 3.7 mmol) and bromoacetonitrile (0.45g, 3.7 mmol) were added to a stirred solution of the sulphoxide (1g, 2 mmol) in DMF at 0°C under a blanket of nitrogen. Stirring was continued for 2 hours after which time tlc indicated the conversion of ester **(202)** to baseline material which did not contain any β-lactam ring products according to ir. The reaction was therefore abandoned.

Synthesis of Diphenylmethyl (6R,7R) 3-Methyl-4β-cyanomethyl-7β-phenoxy-acetamidoceph-3-em-4α-carboxylate 1β-Oxide (459) and diphenylmethyl (6R,7R) 2α-cyanomethyl-3-methyl-7β-phenoxyacetamidoceph-3-em-4-carboxylate 1β-oxide (460) using Bromoacetonitrile.

A solution of the sulphoxide **(202)** (1.33g, 2.51 mmol) in DMF (40 ml) was stirred with potassium *t*-butoxide (0.42g, 3.76 mmol) and bromoacetonitrile (0.9g, 7.5 mmol) for 10 hr at ice temperature. After which time tlc indicated that the majority of sulphoxide **(202)** remained unreacted, thus, more potassium *t*-butoxide (0.42g, 3.76 mmol) and bromoacetonitrile (0.88g, 7.33 mmol) were added and stirring continued for a further 15hr. The reaction mixture was then poured onto ice/water and extracted with ethyl acetate. The ethyl acetate layer was washed with dil HCl, saturated aqueous $NaHCO_3$, brine; dried ($MgSO_4$) and the solvent removed under reduced pressure. Chromatography afforded ***diphenylmethyl (6R,7R) 3-methyl-4β-cyanomethyl-7β-phenoxyacetamido-cep-2-em-4α-carboxylate 1β-oxide (459)***, (0.45g, 31%) with data (ir & nmr) identical as before and ***diphenylmethyl (6R,7R) 2α-cyanomethyl-3-methyl-***

7β-phenoxyacetamidoceph-3-em-4-carboxylate 1β-oxide (460) as a light brown oil (0.12g, 8%); υ_{max} 3376, 2250, 1795, 1742 and 1694 cm^{-1}; δ (250 MHz) 2.06 (3H, s, 3-CH_3), 3.04 (1H, d, J=9.6 Hz, CH_2CN), 3.21 (1H, d, J=4.3 Hz, CH_2CN), 4.35 (1H, dd, J=4.3 & 9.6 Hz, 2-H), 4.71 (2H, s, CH_2CON), 5.1 (1H, d, J=4.85 Hz, 6-H), 6.19 (1H, dd, J=4.8 & 9.5 Hz, 7-H), 6.94-7.51 (16H, m, PhO & CHPh) and 8.23 (1H, d, J=9.6 Hz, N-H); (Found: C,64.99; H,4.94; N,7.31; S,5.57. $C_{31}H_{27}N_3O_6S$ requires C,65.36; H,4.74; N,7.39; S,5.53%); m/e 593 MNa^+.

Starting material **(202)** (0.35g) was also recovered.

Reaction of Diphenylmethyl (6R,7R) 3-Methyl-7β-phenoxyacetamido-ceph-3-em-4-carboxylate 1β-Oxide (202) with Iodoacetonitrile.

A solution of the sulphoxide **(202)** (1.3g, 2.5 mmol); potassium *t*-butoxide (0.42g, 3.74 mmol) and iodoacetonitrile (0.49g, 2.5 mmol) in DMF (20 ml) was stirred for 24 hr at 0°C in a nitrogen atmosphere. The reaction mixture was poured onto ice/water; extracted with ethyl acetate; washed with dil HCl, brine, saturated aqueous $NaHCO_3$, brine; dried ($MgSO_4$) and evaporated *in vacuo*. Chromatography gave only mixed fractions that, according to tlc, corresponded to ***diphenylmethyl (6R,7R) 3-methyl-4β-cyanomethyl-7β-phenoxyacetamido-cep-2-em-4α-carboxylate 1β-oxide (459)*** and ***diphenylmethyl (6R,7R) 2α-cyanomethyl-3-methyl-7β-phenoxyacetamidoceph-3-em-4-carboxylate 1β-oxide (460)***.

Synthesis of Diphenylmethyl (6R,7R) 3-Methyl-4β-cyanomethyl-7β-phenoxy-acetamidoceph-3-em-4α-carboxylate 1β-Oxide (459) and diphenylmethyl (6R,7R) 2α-cyanomethyl-3-methyl-7β-phenoxyacetamidoceph-3-em-4-carboxylate 1β-oxide (460) using Iodoacetonitrile.

The sulphoxide **(202)** (1.1g, 2.1 mmol) was dissolved in DMF (20 ml) and cooled to 0°C under a blanket of nitrogen. Sodium hydride (0.058g, 2.4 mmol)

was added followed by iodoacetonitrile (0.38g, 2.3 mmol) and stirring was continued for 15 hr. According to tlc, the majority of starting material remained unreacted hence sodium hydride (0.05g, 2 mmol) and iodoacetonitrile (0.38g, 2.3 mmol) were added. After 5 hr the reaction solution was poured onto ice/water and extracted with ethyl acetate. The organic layer was washed with dil HCl, brine, saturated aqueous $NaHCO_3$, brine; dried ($MgSO_4$) and evaporated *in vacuo*. Chromatography gave ***diphenylmethyl (6R,7R) 3-methyl-4β-cyanomethyl-7β-phenoxyacetamidocep-2-em-4α-carboxylate 1β-oxide (459)*** (0.31g, 34%); ***diphenylmethyl (6R,7R) 2α-cyanomethyl-3-methyl-7β-phenoxyacetamido-ceph-3-em-4-carboxylate 1β-oxide (460)*** (0.43g, 47%) and recovered sulphoxide **(202)** (0.25g). Analytical and spectroscopic data were identical to previous products obtained.

Attempted Reaction of Diphenymethyl (6R,7R) 3-Methyl-7β-phenoxy-acetamidoceph-3-em-4-carboxylate 1β-Oxide (202) with Iodoacetonitrile in DMSO.

Sodium hydride (0.14g, 5.66 mmol) and iodoacetonitrile (0.95g, 5.66 mmol) were added to a suspension of the sulphoxide **(202)** (2.5g, 4.7 mmol) in DMSO (40 ml) at room temperature. The reaction mixture solidified and DMF (50 ml) was introduced. After 3 hr, tlc indicated numerous products therefore reaction solution was poured onto ice/water; extracted with ethyl acetate; washed with dil HCl, brine, saturated aqueous $NaHCO_3$, brine; dried ($MgSO_4$) and evaporated under reduced pressure. Products were inseparable by column chromatography and the reaction mixture was discarded.

Attempted Reaction of Diphenylmethyl (6R,7R) 3-Methyl-7β-phenoxy-acetamidoceph-3-em-4-carboxylate 1β-Oxide (202) with Benzyl Chloride.

Benzyl chloride (0.38g, 3 mmol) followed by sodium hydride (0.05g, 2 mmol) were added to an ice temperature solution of the sulphoxide **(202)** (0.5g, 1 mmol) dissolved in DMF (20 ml). After 5 hr, the starting material remained unreacted and after 15 hr a complex reaction mixture had formed according to tlc so reaction was abandoned.

Attempted Reaction of Diphenylmethyl (6R,7R) 3-Methyl-7β-phenoxy-acetamidoceph-3-em-4-carboxylate 1β-Oxide (202) with Bromoethyl Ethyl Ether using Triethylamine.

Triethylamine (0.34g, 3 mmol) and bromoethyl ethyl ether (5 ml) were added to a solution of the sulphoxide **(202)** (0.5g, 1 mmol) in DMF (5 ml). Stirring was continued for 24 hr after which time only starting material was present according to tlc analysis therefore the starting material was recovered and the reaction abandoned.

Attempted Reaction of Diphenylmethyl (6R,7R) 3-Methyl-7β-phenoxy-acetamidoceph-3-em-4-carboxylate 1β-Oxide (202) with Bromoethyl Ethyl Ether using Sodium Hydride.

A solution of the sulphoxide **(202)** (0.5g, 1 mmol) in DMF (15 ml) at 0°C was stirred with sodium hydride (0.03g, 1.2 mmol) and bromoethyl ethyl ether (0.19g, 1.2 mmol). After 24 hr of stirring starting material remained unreacted as indicated by tlc. A further 24 hr and the reaction mixture appeared mostly baseline material (tlc) and no carbonyl stretching frequency for the β-lactam ring was observed according to ir so the reaction mixture was discarded.

Attempted Reaction of Diphenylmethyl (6R,7R) 3-Methyl-7β-phenoxy-acetamidoceph-3-em-4-carboxylate 1β-Oxide (202) with Bromoethyl Ethyl Ether using Potassium t-Butoxide.

The sulphoxide **(202)** (0.5g, 1 mmol) was dissolved in DMF (20 ml) and stirred at 0°C under a nitrogen blanket. Bromoethyl ethyl ether (2 ml) and potassium *t*-butoxide (0.22g, 1.9 mmol) in DMF (2 ml) were added to the stirred solution. After 20 hr tlc appeared streaked and ir indicated degradation of the β-lactam ring had occurred therefore reaction was abandoned.

Attempted Reaction of Diphenylmethyl (6R,7R) 3-Methyl-7β-phenoxy-acetamidoceph-3-em-4-carboxylate 1β-Oxide (202) with Bromoethyl Ethyl Ether using Sodium Hydride.

Sodium hydride (0.05g, 2 mmol) and bromoethyl ethyl ether (2 ml) were added to a stirred solution of the sulphide (0.5g, 1 mmol) in DMF (15 ml) at 0°C. The temperature was raised to room temperature after 30 minutes and stirring continued for 15 hr. Tlc indicated a complex mixture of products inseparable by column chromatography.

Attempted Reaction of Diphenylmethyl (6R,7R) 3-Methyl-7β-phenoxy-acetamidoceph-3-em-4-carboxylate 1β-Oxide (202) with Chloroacetone using Sodium Hydride.

Sodium hydride (0.05g, 2.1 mmol) and chloroacetone (0.2, 2.1 mmol) were added to a stirred solution of the sulphoxide **(202)** (1.0g, 2 mmol) in DMF (45 ml) at 0°C under a nitrogen blanket. After stirring for 24 hr the sulphoxide **(202)**, was still present according to tlc therefore sodium hydride (0.07g, 2.8 mmol) and chloroacetone (0.2g, 2.1 mmol) were added. No reaction had occurred after 24 hr and the starting material was recovered unreacted.

Attempted Reaction of Diphenylmethyl (6R,7R) 3-Methyl-7β-phenoxy-acetamidoceph-3-em-4-carboxylate 1β-Oxide (202) with Chloroacetone using Sodium Hydride.

To a stirred solution of the sulphoxide **(202)** (0.5g, 1 mmol) in DMF (20 ml) at 0°C, sodium hydride (0.025g, 1 mmol); chloroacetone (5 ml) and potassium iodide (0.2g) were added. After 24 hr, tlc indicated no reaction had taken place therefore reaction was abandoned.

Attempted Reaction of Diphenylmethyl (6R,7R) 3-Methyl-7β-phenoxy-acetamidoceph-3-em-4-carboxylate 1β-Oxide (202) with p-Methoxybenzyl Chloride.

The ester **(202)**, (0.5g, 1 mmol) was dissolved in DMF and cooled to 0°C in a nitrogen atmosphere. Sodium hydride (0.03g, 1.2 mmol) and *p*-methoxybenzyl chloride (0.19g, 1.2 mmol) were added and stirring continued for 24 hr. According to tlc, starting material remained unreacted and reaction mixture was discarded.

Attempted Reaction of Diphenylmethyl (6R,7R) 3-Methyl-7β-phenoxy-acetamidoceph-3-em-4-carboxylate 1β-Oxide (202) with p-Nitrobenzyl Chloride.

The sulphoxide **(202)** (0.5g, 1 mmol) was dissolved in DMF (20 ml) and cooled to 0°C under a blanket of nitrogen. *p*-Nitrobenzyl chloride (0.21g, 1.2 mmol) was added followed by sodium hydride (0.03g, 1.2 mmol). After 15 hr, the reaction mixture was poured onto ice/water and extracted with ethyl acetate. The organic portion was washed with dil HCl, brine, saturated aqueous $NaHCO_3$, brine; dried ($MgSO_4$) and evaporated *in vacuo* to give a complex mixture of products. Attempted chromatography gave only the sulphoxide **(202)**, (0.1g) and the remaining reaction mixture was discarded.

Attempted Reaction of Diphenylmethyl (6R,7R) 3-Methyl-7β-phenoxy-acetamidoceph-3-em-4-carboxylate 1β-Oxide (202) with Phenacyl Bromide.

The sulphoxide **(202)** (1.5g, 3 mmol) was dissolved in DMF (30 ml) under a nitrogen blanket and cooled to 0°C. Sodium hydride (0.14g, 6 mmol) was added followed by phenacyl bromide (1.19g, 6 mmol) and stirring was continued for 5 hr. The reaction mixture was poured onto ice/water; extracted with ethyl acetate; washed with dil HCl, brine, saturated aqueous $NaHCO_3$, brine; dried ($MgSO_4$) and evaporated under reduced pressure to give an oil (3.25g) with no β-lactam ring containing products. Thus the reaction was abandoned.

Attempted Reaction of Diphenylmethyl (6R,7R) 3-Methyl-7β-phenoxy-acetamidoceph-3-em-4-carboxylate 1β-Oxide (202) with Phenacyl Bromide.

A solution of the sulphoxide **(202)** (1.5g, 3 mmol) was stirred with sodium hydride (0.14g, 6 mmol) in DMF (40 ml) at ice temperature. A dropwise solution of phenacyl bromide (1.19g, 6 mmol) in DMF (20 ml) was added over a period of 15 minutes. After 5 hr, tlc of the reaction mixture indicated numerous products which were inseparable by chromatography. Ir spectroscopy indicated β-lactam ring still intact but as a result of the numerous products present the reaction mixture was discarded.

Attempted Reaction of Diphenylmethyl (6R,7R) 3-Methyl-7β-phenoxy-acetamidoceph-3-em-4-carboxylate 1β-Oxide (202) with Phenacyl Bromide.

The sulphoxide **(202)** (1.5g, 3 mmol) was dissolved in DMF (40 ml) and cooled to 0°C. Sodium hydride (0.08g, 3.2 mmol) was added followed 2 minutes later by phenacyl bromide (0.64g, 3.2 mmol) in DMF (20 ml) dropwise over a period of 2 hr. After a further 3 hr of stirring the reaction mixture was poured onto ice/water and extracted with ethyl acetate. The organic layer was washed with dil HCl, brine, saturated aqueous $NaHCO_3$, brine, dried ($MgSO_4$) and concentrated

under reduced pressure to give an oil (1.75g). Column chromatography isolated only the sulphoxide **(202)** (0.93g) and ir of the remaining reaction mixture indicated the lack of the β-lactam carbonyl stretching frequency and as a result the reaction was abandoned.

Attempted Reaction of Diphenylmethyl (6R,7R) 3-Methyl-7β-phenoxy-acetamidoceph-3-em-4-carboxylate 1β-Oxide (202) with Phenacyl Chloride.

A solution of the sulphoxide **(202)** (0.5g, 1 mmol) in DMF (20 ml) was cooled to 0°C in a nitrogen atmosphere. Sodium hydride (0.04g, 1.13 mmol) was added followed by phenacyl chloride (0.44g, 2.85 mmol) dissolved in DMF (5 ml). Four hours later tlc indicated majority of starting material was still present, hence sodium hydride (0.04g, 1.7 mmol) was added and stirring continued overnight. The reaction mixture was poured onto ice/water; extracted with ethyl acetate; washed with dil HCl, brine, saturated aqueous $NaHCO_3$, brine; dried ($MgSO_4$) and evaporated *in vacuo* to give a brown oil that contained no β-lactam carbonyl stretch (~1780 cm^{-1}) according to ir. Hence the reaction mixture was discarded.

Preparation of Diphenylmethyl (6R,7R) 2-(2'-cyanoethylene)-3-methyl-7β-phenoxyacetamidoceph-3-em-4-carboxylate (482).

Trifluoroacetic anhydride (1.8 ml) was stirred at room temperature with acetic anhydride (3 ml) for 3.5 hr. The sulphoxide **(368)** (0.6g, 1 mmol) dissolved in dichloromethane (10 ml) was added followed by 2,6-lutidine (0.2 ml, 2 mmol) and stirring continued overnight. Ethyl acetate was added and the solution was washed with saturated aqueous $NaHCO_3$, 20% H_3PO_4, brine, dried ($MgSO_4$) and evaporated *in vacuo*. Column chromatography afforded the major product ***diphenylmethyl (6R,7R) 2-(2'-cyanoethylene)-3-methyl-7β-phenoxy-acetamidoceph-3-em-4-carboxylate (482)*** as an oil (0.23g, 40%); υ_{max}, 3327, 2250, 1782, 1724 and 1695 cm^{-1}; δ (250 MHz) 2.16 (3H, s, 3-CH_3), 3.29 (2H, ddd,

J=6.3, 7.5 & 19 Hz, CH_2CN), 4.59 (2H, s, CH_2CON), 5.08 (1H, d, J=4.5 Hz, 6-H), 5.90 (1H, dd, J=4.5 & 8.8 Hz, 7-H), 6.19 (1H, dd, J=6.3 & 7.5 Hz, 2-C<u>H</u>CH_2CN) and 6.91-7.45 (17H, m, PhO, $CHPh_2$ & N-H); (Found: C,67.98; H,4.92; N,7.26; S,5.40. $C_{32}H_{27}N_3O_5S$ requires C,67.94; H,4.81; N,7.43; S,5.67); m/e 583 MNH_4^+ and 566 MH^+.

Preparation of Diphenylmethyl (6R,7R) 2-(2'-t-Butyloxycarbonylmethylene)-3-methyl-7β-phenoxyacetamidoceph-3-em-4-carboxylate 1β-oxide (483) Via a Pummerer Rearrangement.

Acetic anhydride (3 ml) and trifluoroacetic anhydride (1.8 ml) were stirred at room temperature under a blanket of nitrogen for 2 hr. This solution was cooled to 0°C and the sulphoxide **(436)** (0.5g, 0.8 mmol) and 2,6-lutidine (0.16 ml, 1.6 mmol) were added. After 17 hr, ethyl acetate was added and the solution was washed with saturated aqueous $NaHCO_3$, 20% H_3PO_4, brine; dried ($MgSO_4$) and evaporated under reduced pressure. Separation by column chromatography afforded ***diphenylmethyl (6R,7R) 2-(2'-t-butyloxycarbonylmethylene)-3-methyl-7β-phenoxyacetamidoceph-3-em-4-carboxylate (483)*** as an oil (0.36g, 74%); υ_{max} 3372, 1787, 1731 and 1691 cm^{-1}; δ (250 MHz) 1.47 (3H, s, 3-CH_3), 1.51 (9H, s, CMe_3), 4.59 (2H, s, CH_2CON), 5.08 (1H, d, J=4.6 Hz, 6-H), 5.97 (1H, dd, J=4.6 & 10.5 Hz, 7-H), 6.26 (1H, s, 2-$CHCO_2$) and 6.91-7.49 (17H, m, PhO, $CHPh_2$ & N-H); (Found: C,66.87; H,5.34; N,4.22; S,5.29. $C_{35}H_{34}N_2O_7S$ requires C,67.07; H,5.47; N,4.47; S,5.12.); m/e 626 M^+, 627 MH^+, 650 MNa^+ and 666 MK^+.

Attempted Pummerer Rearrangement of mixture containing Diphenylmethyl (6R,7R) 2,4β-Dibenzyl-3-methyl-7β-phenoxyacetamidoceph-2-em-4α-carboxylate 1β-Oxide (431) and suspected Diphenylmethyl (6R,7R) 2-Benzyl- 3-methyl-7β-phenoxyacetamidoceph-3-em-4-carboxylate 1β-Oxide (432).

Triflouroacetic anhydride (3.6 ml) and acetic anhydride (6 ml) were stirred under nitrogen at room temperature for 3 hr. A mixture of the di-adduct **(431)** and suspected mono-adduct **(432)** (2.49g) was dissolved in dichloromethane (30 ml) and added with 2,6-lutidine (0.47 ml, 4 mmol) at 0°C. Stirring was continued overnight after which time tlc indicated some starting material remained unreacted. Ethyl acetate was added and the solution washed with saturated aqueous $NaHCO_3$, 20% H_3PO_4, brine; dried ($MgSO_4$); evaporated under reduced pressure and chromatographed to give ***diphenylmethyl (6R,7R) 3-methyl-4β-benzyl-7β-phenoxyacetamidoceph-3-em-4α-carboxylate 1β-oxide (485)*** as an oil (1.01g); υ_{max} 3402, 1781, 1740 and 1700 cm^{-1}. δ (250 MHz) 1.78 (3H, d, J=0.84 Hz, $3CH_3$), 3.35 & 3.92 (2H, ABq, J=42.4 Hz, 4-CH_2Ph) 4.49-4.62 (3H, m, CH_2CON & 6-H), 5.61 (1H, dd, J=4.0 & 8.8 Hz, 7-H), 6.09 (1H, d, J=0.84 Hz, 2-H) and 6.93-7.40 (22H, m, PhO, $CHPh_2$, 4-CH_2Ph & N-H); (Found: C,69.88; H,5.03; N,4.29; S,4.95. $C_{36}H_{32}N_2O_6S$ requires C,69.65; H,5.19; N,4.51; S,5.16); m/e 621 MH^+ and 638 MNH_4^+.

Also isolated was ***diphenylmethyl (6R,7R) 3-methyl-4α-benzyl-7β-phenoxyacetamidoceph-3-em-4β-carboxylate 1β-oxide (486)*** as an oil (0.35g); υ_{max}3351, 1778, 1735 and 1698 cm^{-1}; δ (250 MHz) 1.61 (3H, s, 3-CH_3), 2.16 (1H, s, 2-H), 3.94 & 4.11 (2H, ABq, J=40.4 Hz, 4-CH_2Ph), 4.44 (1H, d, J=4.7 Hz, 6-H), 4.54 & 4.55 (2H, ABq, J=18 Hz*, CH_2CON), 5.80 (1H, dd, J=4.7 & 10.4 Hz, 7-H),6.54 (1H, s, 2-H), 6.96-7.41 (21H, m, PhO, $CHPh_2$ & 4-CH_2Ph) and 8.24 (1H, d, J=10.4 Hz, N-H); (Found: C,69.44; H,5.02; N,4.22; S,4.85. $C_{36}H_{32}N_2O_6S$ requires C,69.65; H,5.19; N,4.51; S,5.16; m/e 621 MH^+ and 638 MNH_4^+.

Attempted Pummerer Rearrangement of Diphenylmethyl (6R,7R) 2α-Cyanomethyl-3-methyl-7β-phenoxyacetamidoceph-3-em-4-carboxylate 1β-Oxide (460).

Trifluoroacetic anhydride (1.8 ml) and acetic anhydride (3 ml) were stirred in an nitrogen atmosphere at room temperature for 3 hr. The mixture was cooled to 0°C and the 2-cyanomethyl adduct **(460)** (0.42g, 0.74 mmol) dissolved in dichloromethane (15 ml) was added followed by 2,6-lutidine (0.15 ml, 1.5 mmol). Stirring was continued for 15 hr after which time tlc indicated a complex reaction mixture inseparable by chromatography and thus, reaction was abandoned.

Attempted Pummerer Rearrangement of Diphenylmethyl (6R,7R) 2α-Diphenyl-methyl-3-methyl-7β-phenoxyacetamidoceph-3-em-4-carboxylate 1β-Oxide (435).

Trifluoroacetic anhydride (1.8 ml) and acetic anhydride (3 ml) were stirred in an nitrogen atmosphere at room temperature for 4 hr. The solution was cooled to 0°C and the sulphoxide **(435)** (0.51g, 0.72 mmol) in dichloromethane (15 ml) was added followed by 2,6-lutidine (0.15 ml). Stirring was continued overnight after which time tlc indicated starting material remained unreacted therefore 2,6-lutidine (0.15 ml) was added and stirring continued for 24 hr. Ethyl acetate was added to reaction mixture, which was then washed with saturated aqueous $NaHCO_3$, 20% H_3PO_4, brine; dried ($MgSO_4$) and evaporated *in vacuo* to give a complex mixture of products inseparable by column chromatography. Thus, the reaction mixture was discarded.

Attempted Reaction of Ethyl Chloroformate with Diphenylmethyl (6R,7R) 2-(2'-t-Butyloxycarbonylmethyl)-3-methyl-7β-phenoxyacetamidoceph-3-em-4-carboxylate 1β-Oxide (436).

A solution of the sulphoxide **(436)** (0.7g, 1.1 mmol) in dry THF (30 ml) was stirred with ethyl chloroformate (0.35g, 3.3 mmol) and triethylamine (0.33g, 3.3 mmol) at room temperature overnight. The reaction mixture was partitioned between water and ethyl acetate. The organic portion was washed with dil HCl, brine saturated aqueous $NaHCO_3$, brine, dried ($MgSO_4$) and evaporated under reduced pressure. Column chromatography provided a mixture of three products which could not be separated further and the mixture was discarded.

Attempted Elimination of 2-Ethoxy Group from Speculated Diphenylmethyl 2-(2'-t-Butyloxycarbonylmethyl)-2-ethoxy-3-methyl-7β-phenoxyacetamido-ceph-3-em-4-carboxylate Product (489).

The mixture of sulphides (0.1g, 0.15 mmol) dissolved in dry THF was stirred with triethylamine (0.1g, 1 mmol) at ice temperature. After 1.5 hr no reaction had occurred according to tlc and the temperature was raised to room temperature for 4 hr. Tlc indicated a complex reaction mixture inseparable by chromatography and the reaction was abandoned.

Attempted Reaction of Benzaldehyde with Diphenylmethyl (6R,7R) 3-Methyl-7β-phenoxyacetamidoceph-3-em-4-carboxylate 1β-Oxide (202).

The sulphoxide **(202)** (0.5g, 1 mmol) was heated at reflux in benzaldehyde (5 ml) for 1 hr. According to tlc, the starting material had reacted but ir indicated the lack of the β-lactam carbonyl stretching frequency in the product mixture and as a result, the reaction was abandoned.

Attempted Reaction of Benzaldehyde with Diphenylmethyl (6R,7R) 3-Methyl-7β-phenoxyacetamidoceph-3-em-4-carboxylate 1β-Oxide (202).

Zinc chloride (0.025g) was added to a suspension of the sulphoxide **(202)** (0.5g, 1 mmol) in benzaldehyde (2.8 ml). The reaction mixture was heated at 100°C for 1 hr after which time tlc indicated starting material had reacted. Ether was added to the black oily residue and decanted off. Addition of petrol to the combined ether layers gave ***3-methyl-7β-phenoxyacetamidoceph-3-em-4-carboxylic acid (494)*** as an orange solid (0.25g, 69%); υ_{max}3600-2600, 3300, 1793, 1710 and 1675. δ (d_6-DMSO, 60 MHz) 2.08 (3H, s, 3-CH_3), 3.64 (2H, s, 2-H), 4.60 (2H, s, CH_2CON), 4.88 (1H, d, J=4.8 Hz, 6-H), 5.92 1H, dd, J=4.8 & 9.6 Hz, 7-H), 7.04 (5H, m, PhO) 8.00 (1H, d, J=9.6 Hz, N-H) and 10.7 (1H, br s, COOH).

Attempted Reaction of Benzaldehyde with Diphenylmethyl (6R,7R) 3-Methyl-7β-phenoxyacetamidoceph-3-em-4-carboxylate 1β-Oxide (202).

Triethylamine (0.19g, 2 mmol) and acetic anhydride (0.11g, 1.1 mmol) were added to a stirred solution of the sulphoxide **(202)** (0.5g, 1 mmol) in benzaldehyde (5 ml) under a blanket of nitrogen. The reaction mixture was heated at 100°C for 1.5 hr, poured onto water and extracted with ethyl acetate. The organic phase was washed with dil HCl, brine, saturated aqueous $NaHCO_3$, brine, dried ($MgSO_4$) and evaporated *in vacuo*. Numerous products were observed (tlc) and ir indicated the presence of β-lactam carbonyl containing products. An attempt at separation by chromatography failed and the reaction mixture was discarded.

Attempted De-oxygenation of Diphenylmethyl (6R,7R) 2α,4β-(3'-Oxobutyl)-3-methyl-7β-phenoxyacetamidoceph-2-em-4α-carboxylate 1β-Oxide (383).

Acetyl chloride (0.15g, 2 mmol) and potassium iodide (0.26g, 1.5 mmol) were added to a solution of the di-adduct **(383)** (0.35g, 0.5 mmol) in DMF (20 ml) at 0°C. Stirring was continued overnight after which time no reaction had taken place according to tlc and reaction was abandoned.

De-oxygenation of Diphenylmethyl (6R,7R) 2α,4β-(3'-Oxobutyl)-3-methyl-7β-phenoxyacetamidoceph-2-em-4α-carboxylate 1β-Oxide (383).

A solution of the di-adduct **(383)** (0.1g, 0.15 mmol) in DMF (10 ml) at 0°C was stirred for 35 minutes with PBr (0.3 ml). The reaction mixture was poured onto saturated aqueous $NaHCO_3$ and extracted with ethyl acetate. The organic portion was washed with saturated aqueous $NaHCO_3$, brine, dried ($MgSO_4$) and evaporated *in vacuo* to give ***diphenylmethyl (6R,7R) 2α,4β-(3'-oxobutyl)-3-methyl-4α-carboxylate (495)*** as a yellow oil (0.06g, 61%); υ_{max} 3309, 1771, 1740 and 1714 cm^{-1}; δ (250 MHz) 1.63 (3H, s, 3-CH_3), 2.12 (3H, s, $COCH_3$), 2.14 (3H, s, $COCH_3$), 2.39-2.96 (8H, m, CH_2CH_2CO), 4.57 (2H, s, CH_2CON), 5.16 (1H, d, J=4.4 Hz, 6-H) 5.43 (1H, dd, J=4.4 & 8.5 Hz, 7-H) and 6.88-7.37 (17H, m, PhO, $CHPh_2$ & N-H); (Found: C,69.97; H,5.76; N,3.97. $C_{37}H_{38}N_2O_7S$ requires C,67.87; H,5.85; N,4.28); m/e 655 MH^+.

De-oxygenation of Diphenylmethyl 2-Diethyl Methylenemalonate-3-methyl-7β-phenoxyacetamidoceph-3-em-4-carboxylate 1β-Oxide (390).

The sulphoxide **(390)** (1.04g,1.4 mmol) in dry DMF (30 ml) was stirred at ice temperature with acetyl chloride (0.34g, 4.3 mmol) and potassium iodide (0.71g, 4.3 mmol) for 10 hr. The reaction mixture was poured onto ice/water/$Na_2S_2O_5$ and extracted with ethyl acetate. The organic phase was washed with saturated aqueous $NaHCO_3$ solution, brine, dried ($MgSO_4$) and solvent

removed under reduced pressure. Chromatography afforded one major product which was identified as ***diphenylmethyl (6R,7R) 2-diethyl methylenemalonate 3-methyl-7β-phenoxyacetamidoceph-3-em-4-carboxylate (496)*** as a yellow solid (0.65g, 64%); υ_{max} 3323, 1785, 1731 and 1599 cm^{-1}; δ (250 MHz) 1.25 (6H, t, J=7.2 Hz, $CH_2C\underline{H}_3$), 2.2 (3H, s, 3-CH_3), 4.18 (4H, q, J=7.2 Hz, $C\underline{H}_2CH_3$), 4.55 (2H, s, CH_2CON), 4.60 (1H, d, J=9.6 Hz, CH-C$\underline{H}(CO_2Et)_2$), 5.05 (1H, d, J=4.8 Hz, 6-H), 5.9 (1H, dd, J=4.8 & 9.0 Hz, 7-H), 6.55 (1H, d, J=9.6 Hz, 2-C$\underline{H}$-CH) and 6.95-7.4 (17H, m, PhO, $CHPh_2$ & N-H); (Found: C,64.88; H,5.31; N,4.17; S4.43. $C_{37}H_{36}N_2O_9S$ requires C,64.9; H,5.3; N,4.09; S,4.68); m/e 708.4 MNa^+.

Attempted De-oxygenation of Diphenylmethyl (6R,7R) 2α-Ethoxy-3-methyl-7β-phenoxyacetamidoceph-3-em-4-carboxylate 1β-Oxide (392).

Phosphorus tribromide (0.3 ml) was added to a stirred solution of the sulphoxide **(392)** (0.15g, 0.3 mmol) in dry DMF (10 ml) at ice temperature for 30 min. The reaction mixture was poured onto a saturated solution of $NaHCO_3$ and extracted with ethyl acetate . The organic layer was washed with saturated aqueous $NaHCO_3$, brine, dried ($MgSO_4$) and evaporated *in vacuo*. Attempted separation by column chromatography did not yield any homogeneous products and the reaction was discarded.

De-oxygenation of Diphenylmethyl (6R,7R) 2α-Cyanocyclopropyl-3-methyl-7β-phenoxyacetamidoceph-3-em-4-carboxylate 1β-Oxide (382a).

Phosphorus tribromide (1.2 ml) was added to a stirred solution of the sulphoxide **(382a)** (0.98g, 1.7 mmol) in dry DMF (20 ml) at ice temperature. After 5 hr the reaction mixture was poured onto a saturated solution of $NaHCO_3$ and partitioned by ethyl acetate. The organic portion was washed with saturated aqueous $NaHCO_3$, brine, dried ($MgSO_4$) and evaporated *in vacuo*. Chromatography afforded ***diphenylmethyl (6R,7R) 2α-cyanocyclopropyl-***

3-methyl-7β-phenoxyacetamidoceph-3-em-4-carboxylate (497a) as a brown oil (0.17g, 17%); υ_{max} 3324, 2240, 1788 and 1725 cm^{-1}; δ (250 MHz) 1.65 (3H, s, 3-CH_3), 2.08-2.11 (3H, m, 2-CH_2CHCN), 4.55 (2H, s, CH_2CON), 5.26 (1H, d, J=4.8 Hz, 6-H), 6.10 (1H, dd, J=4.8 & 9.7 Hz, 7-H) and 6.96-7.44 (17H, m, PhO, $CHPh_2$ & N-H). (Found: C,67.87; H,5.01; N,7.04; S,5.41. $C_{32}H_{27}N_3O_5S$ requires C,67.96; H,4.78; N,7.43; S,5.66%); m/e 583 MNH_4^+.

De-oxygenation of Diphenylmethyl (6R,7R) 2α-Cyanocyclopropyl-3-methyl-7β-phenoxyacetamidoceph-3-em-4-carboxylate 1β-Oxide (382b).

A solution of the sulphoxide **(382b)** (1.12g, 2 mmol) in dry DMF (20 ml) was stirred at 0°C with phosphorus tribromide (0.6 ml) for 2 hr. The reaction mixture was poured onto saturated aqueous $NaHCO_3$ and extracted with ethyl acetate; washed with saturated aqueous $NaHCO_3$, brine; dried ($MgSO_4$); concentrated *in vacuo* and chromatographed to give ***diphenylmethyl (6R,7R) 2α-cyanocyclopropl-3-methyl-7β-phenoxyacetamidoceph-3-em-4-carboxylate (497b)*** as a brown oil (0.73g, 67%); υ_{max} 3350, 2238, 1777, 1724 and 1690 cm^{-1}; δ (250 MHz) 1.73 (3H, s, 3-CH_3), 1.84-1.86 (2H, m, 2-C<u>H</u>$_2$CHCN), 2.06-2.13 (1H, m, 2-CH_2C<u>H</u>CN), 4.53 (2H, s, CH_2CON), 4.96 (1H, d, J=2.0 Hz, 6-H), 5.20 (1H, dd, J=2.0 & 8.15 Hz, 7-H), 6.90-7.43 (16H, m, PhO & $CHPh_2$) and 7.50 (1H, d, J=8.15 Hz, N-H); (Found: C,67.93; H,4.94; N,7.09; S,5.49. $C_{32}H_{27}N_3O_5S$ requires C,67.96; H,4.78; N,7.43; S,5.66%); m/e 583 MNH_4^+.

Attempted De-esterification of Diphenylmethyl (6R,7R) 2α-(2'-t-Butyl-oxycarbonylmethylene)-3-methyl-7β-phenoxyacetamidoceph-3-em-4-carboxylate (483).

To a solution of the sulphide **(483)** (0.43g, 0.7 mmol) in dichloromethane (10 ml) and anisole (2.5 ml) at 0°C was added trifluoroacetic acid (3.8 ml). After stirring for 30 minutes the solvent was evaporated under reduced

pressure and the residue dissolved in ethyl acetate. The product was partitioned with saturated aqueous $NaHCO_3$ and washed with ethyl acetate. The $NaHCO_3$ layer was acidified to pH 2 with 5M HCl and the product extracted into ethyl acetate. The organic phase was washed with brine, dried ($MgSO_4$) and evaporated *in vacuo* to give products of degradation as indicated by the lack of β-lactam carbonyl stretching frequency on ir and so the reaction mixture was discarded.

Attempted De-esterification of Diphenylmethyl (6R,7R) 2α-(2'-Cyanoethylene)-3-methyl-7β-phenoxyacetamido-ceph-3-em-4-carboxylate (482).

The sulphide **(482)** (0.35g, 0.6 mmol) was dissolved in dichloromethane (10 ml) and anisole (2.5 ml). The solution was cooled to 0°C and trifluoroacetic acid (3.8 ml) was added. Stirring was continued for 30 minutes; the solvent was evaporated *in vacuo* and the oily residue dissolved in ethyl acetate. The product was extracted into $NaHCO_3$ solution and extract washed with ethyl acetate. The aqueous portion was acidified to pH 2 with 5M HCl and product extracted into ethyl acetate. The organic phase was washed with brine, dried ($MgSO_4$) and evaporated under reduced pressure to give a brown oil which did not contain the β-lactam carbonyl according to ir. Thus, the reaction was abandoned.

Attempted De-esterification of Diphenylmethyl (6R,7R) 2-Diethyl Methylenemalonate-3-Methyl-7β-phenoxyacetamidoceph-3-em-4-carboxylate (483)

To a solution of the sulphide **(496)** (0.43g, 0.6 mmol) in dichloromethane (10 ml) and anisole (2.5 ml) at 0°C was added trifluoroacetic acid (3.8 ml). After stirring for 30 minutes the solvent was evaporated under reduced pressure and the residue dissolved in ethyl acetate. The product was partitioned with saturated aqueous $NaHCO_3$ and washed with ethyl acetate. The $NaHCO_3$ layer was acidified to pH 2 with 5M HCl and the product extracted into ethyl

acetate. The organic phase was washed with brine, dried ($MgSO_4$) and evaporated *in vacuo* to give products of degradation as indicated by the lack of β-lactam carbonyl stretching frequency on ir and so the reaction mixture was discarded.

De-esterification and Decarboxylation of Diphenylmethyl (6R,7R) 2α,4β-Dibenzyl-3-methyl-7β-phenoxyacetamidoceph-2-em-4α-carboxylate 1β-Oxide (431).

A solution of the sulphoxide **(431)** (0.5g, 0.7 mmol) in dichloromethane (5 ml) was stirred with anisole (2.5 ml) and trifluoroacetic acid (3.8 ml) at room temperature for 1 hr. Dichloromethane was evaporated *in vacuo* and the oily residue was redissolved in ethyl acetate (15 ml) and stirred overnight with triethylamine (1 ml). Ethyl acetate (100 ml) was added and the solution was washed with dil HCl, brine, saturated aqueous $NaHCO_3$, brine, dried ($MgSO_4$) and concentrated under reduced pressure. Chromatography isolated one major product ***2α,4β-dibenzyl-3-methyl-7β-phenoxyacetamidoceph-2-em 1β-oxide (500)*** as an oil (0.17g, 48%); υ_{max} 3325, 1783 and 1693 cm^{-1}; δ (250 MHz) 1.97 (3H, s, 3-CH_3), 2.77-3.12 (2H, m, 2-CH_2Ph), 3.65-3.67 (1H, m, 2-H), 3.88 (1H, d, J=4.8 Hz, 6-H), 3.97 (1H, d, J=15.5 Hz, 4-CH_2Ph), 4.26 (1H, d, J=15.5 Hz, 4-CH_2Ph), 4.52 (2H, s, CH_2CON), 5.91 (1H, dd, J=4.8 & 10.5 Hz, 7-H), 6.88-7.33 (15H, m, PhO, 2-CH_2Ph & 4-CH_2Ph) and 7.99 (1H, d, J=10.5 Hz, N-H); (Found: C,69.76; H,5.63; N,5.49; S,6.38. $C_{29}H_{28}N_2O_4S$ requires C,69.57; H,5.64; N,5.60; S,6.41); m/e 501 MH^+ and 518 MNH_4^+.

De-eserification and Attempted Decarboxylation of Diphenylmethyl (6R,7R) 2α,2β-Diphenylmethyl-3-methyl-7β-phenoxyacetamidoceph-3-em-4-carboxylate 1β-Oxide (434).

A solution of the sulphoxide **(434)** (0.5g, 0.6 mmol) in dichloromethane (15 ml) with anisole (3.5 ml) and trifluoroacetic acid (3.8 ml) was stirred at room temperature for 1 hr. The solvent was evaporated *in vacuo* and washed with toluene (20 ml). The oily residue was dissolved in ethyl acetate (20 ml) and triethylamine (2 ml) was added. Stirring was continued at room temperature for 24 hr after which time tlc indicated starting material remained unreacted. Attempted recovery of products failed and reaction was abandoned.

* ABq values are estimated as a result of vague nmr spectra data.

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4.0 REFERENCES

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